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Gastroprotective Effect of Bidens pilosa L. Leaves against Indomethacin-Induced Gastric Ulceration in Rats

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ABSTRACT
Using an indomethacin (IND)-induced gastric ulcer model in rats, we sought to identify the phytochemical components of Bidens pilosa (B. pilosa) leaves and examine their gastroprotective effects. There was a total of 56 male albino adult rats used, split into 7 groups (n=8 rats per group). Group (1) was control -ve normal (non- treated) and group (2) was +ve control given orally 30 mg/ kg indomethacin (IND). GROUP (3) B. pilosa extract I + IND and will receive B. pilosa extract (75 mg/kg b.wt /day) orally. Groups (4), (5) and (6) were administered IND plus 75, 150 and 300 mg/kg of B. pilosa extract, respectively. Group (7) was given 30 mg/ kg IND and received Ranitidine as a reference drug (50 mg/kg b.wt /day) orally for 2 ~~2~~ consecutive weeks before IND administration. The experiment concluded with the sacrifice of the rats, the opening of their stomachs, and the collection of blood samples for biochemical examination. Gastric mucosal homogenates were examined for antioxidant enzyme activities. The results showed that dried powder of B. pilosa leaves contains carbohydrates, glycosides, sterols, triterpenoids, flavonoids, tannins, saponins and alkaloids, while Anthraquinones were absent. B. pilosa extract increase body weight gain, increased levels of antioxidant enzymes glutathione peroxidase (GPx), Superoxide dismutase (SOD) & Catalase (CAT) in gastric homogenate and decreased serum liver enzymes. B. pilosa extract alleviated gastric ulceration in rats. Therefore B. pilosa may be beneficial for patients suffering from gastric ulcer.

Keywords: Bidens Pilosa- Gastric ulcer- Indomethacin – Phytochemical analysis, Biochemical parametrs – Histopathlogy.

INTRODUCTION
The risk of death and disability caused by gastric ulcers is high. Upper gastrointestinal bleeding can be caused by a stomach ulcer if it is left untreated [(1)]. Gastroduodenal ulcers are thought to have their origins in a number of factors, including the secretion of acid-pepsin, the presence of parietal cells, the integrity of the mucosal barrier, the creation of mucus, the delivery of blood and nutrients, the regeneration of damaged cells, and the presence of endogenous [(2)].

Peptic ulcers can be caused by a number of different things, including poor eating habits, smoking cigarettes, taking too many nonsteroidal anti-inflammatory drugs, being under a lot of stress, having a family history of the disease, or being infected with Helicobacter pylori, as reported by **Kim *et al.*** [(3)]. While the use of various pharmaceutical medicines has helped reduce mortality and morbidity rates associated with gastroduodenal ulcer and peptic disorders, the risks associated with these treatments make them far from optimal.

Bidens pilosa L. (B. pilosa, Family Asteraceae) is a member of the daisy family also known as black-jack, hairy beggar-ticks, and Spanish needle. Originally from South America, this therapeutic herb is now found in nearly every country [(4)]. B. pilosa is an annual herb that can reach a height of 1.5 m and has stems that are finely hairy. Bright green, toothed & oblong or ovate lanceolate in shape, the leaves are compound with 3–7 imparipinnate leaflets. Flower clusters can be either white or yellow, and the seeds are a long, thin black with prominent ribs [(5)]. Several studies have shown that B. pilosa plant extracts have therapeutic effects, including those of being antiulcerogenic [(6)], antitumor [(7)], immunosuppressive [(8)], hepatoprotective [(9)], and antioxidant [(10, 11)].

MATERIALS AND METHODS
Field-fresh B. pilosa L. leaves, from the village of El-Blakos in the city of Kom Hamada in the governorate of El-Beheira. Leaves cataloged by the Agricultural Research Service's Flora and Phytotaxonomy Department, part of the Horticultural Research Institute.

Drugs and Chemicals: The 100mg capsules of indomethacin were purchased from Miss Scientific Co. in Dokki, Giza. GlaxoSmithKline, Egypt, was the source for our ranitidine. Nitric oxide (NO), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA). Morgan Co., Cairo, Egypt, cellulose, and D-L methionine. El-Gomhoriya Pharm. & Chem. Ind. Co. in Cairo, Egypt, supplied the mineral and vitamin components and the sucrose. Corn oil purchased at a neighborhood store. Starch and Glucose Co. in Helwan, Cairo, Egypt, is where we'll be buying our corn starch.

Experimental Animals: Animals from the Sprague Dawley strain, adult male albino rats (56 totals) were bought from the Animal House at the National Research Center in Dokki, Egypt.

Methods:
Preparation of Plant Extract: B. pilosa leaves were cleaned with running water and then dried in the air. The air-dried leaves were processed into a powder and stored in an airtight container until needed. 500 grams

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of powdered B. pilosa leaves were soaked in a solvent of 700 milliliters of 95% ethanol & 300 milliliters of distilled water at room temperature for 24 hours while being stirred. A double layer of gauze was used to filter the infusion. A rotary evaporator was used to evaporate the filtrates at 40 degrees Celsius in a vacuum [12, 13]. The chemical analysis of dried powdered B. pilosa leaves included the determination of moisture content, total protein, fat, fiber, and ash using the procedures specified in **A.O.A.C, [14],** and the determination of carbohydrates content using the method of difference.

Phytochemical Screening: The phytochemical analysis of B. pilosa leaves extract was performed as described by **Harborne, [15]** at Unit of Pharmacognosy and Chemistry of Medicinal Plants, National Research Center, Dokki, Egypt.

Induction of Gastric Ulcer: regarding the findings of **Bhattacharya *et al*. [16]**, indomethacin (IND) developed gastric ulcers in rats. After fasting for 24 hours, the rats received a single dosage of indomethacin (30 mg/kg) by mouth. Gastric mucosal damage of varying severity was found 4 hours after indomethacin treatment.

Experimental Design: Water was available ad libitum, and the rats were nourished a basal diet prepared regarding [17]. The rats were given a week to adjust before being used in the experiments. Following the adaption phase, the rats were randomly split into seven groups of eight.

This is how the rats were divided up:
Group 1: control group, rats will not be ulcerated or treated.

Group 2: Rats in the IND (non-pretreated) group will receive 30 milligrams of indomethacin per kilogram of body weight (mg/kg b.wt) orally in distilled water. Prior to ulcer induction, rats will go without food for 24 hours but will have access to running water.

Group 3: B. pilosa extract I + IND group, rats will receive B. pilosa extract (75 mg/kg b.wt /day) orally for 2 consecutive weeks before IND administration. This dose was chosen according to the study of **Sabiu *et al*. [18]**.

Group 4: B. pilosa extract II + IND group, rats will receive B. pilosa extract (150 mg/kg b.wt /day) orally for 2 consecutive weeks before IND administration. This dose was chosen according to the study of **Sabiu *et al*. [18]**.

Group 5: B. pilosa extract III + IND group, rats will receive B. pilosa extract (300 mg/kg b.wt /day) orally for 2 consecutive weeks before IND administration.

Group 6: B. pilosa extract IV + IND group, rats will receive B. pilosa extract (450 mg/kg b.wt /day) orally for 2 consecutive weeks before IND administration. **Group 7:** RAN + IND group, rats will receive ranitidine (RAN) as a reference drug (50 mg/kg b.wt /day) orally for 2 consecutive weeks before IND administration. The dose of (RAN was chosen according to the study of **Malfará *et al*. [19]**.

Biological Evaluation: Weekly calculations were made of feed efficiency ratio (FER), feed intake (FI), and body weight percent (BWG %). After 4 hours of receiving IND, the rats were killed while just minimally sedated. To histopathologically analyze mucosal lesions, the stomachs were taken out, opened along the larger curvature, and saline-rinsed. Serum will be centrifuged from blood samples taken from each rat for biochemical examination.

Index of ulceration and inhibition of ulceration as a percentage: After 4 hours after indomethacin (IND) injection, rats were slaughtered and their stomachs were quickly excised, opened along their greater curvature, cleaned with saline, and examined under a microscope for ulcers. Glandular mucosa scoring for quantity and severity of damaged spots. **Sabiu *et al*. [16]** provided the formula for determining the ulcer index, which was as follows: Ulcer index (U.I.) =Mean ulcer score of a group of animals similarly treated X % of ulcerated animals of this group [20].

Serum Biochemical Analysis: Reitman and Frankel (1957) conducted an experiment in which they assessed the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) found in the liver. Following the procedure outlined by **Roy**, enzyme activity was evaluated by measuring the absorbance of a sample using a spectrophotometer (DU 7400) calibrated to 510 nm [21].

Analysis of Gastric Homogenate: The procedures used to quantify the amounts of glutathione peroxidase (GPx), superoxide dismutase (SOD).

Histopathological Examination: Following the documentation of the ulceration, a longitudinal slice of stomach tissue was removed from the top of the stomach for the purpose of being examined by a histologist based on the research carried out by Bancroft and colleagues in 1996.

Statistical Analysis: The data will be displayed using a Mean SE format. One-way analysis of variance (ANOVA) will be utilized to match data, with a suitable post hoc test to establish statistical significance. SPSS version 22 will be used for analysis. Significant results were considered to exist when the p-value was less than 0.05.

Ethical Approval: The research project was given approval by the Ethics Board of Helwan University, and the participants in the trial were provided with

all the information they need regarding the experiment. Each person who took part in the research was required to give their informed consent in writing. This research was carried out on humans in compliance with The Code of Ethics of the World Medical Association, also known as the Declaration of Helsinki for research involving people.

RESULT

Data in table (1) reported that leaves B. pilosa powder contains protein (29.10%), fats (1.48%), carbohydrate (31.54%), crude fibers (8.08%), moisture (11.4%) and ash (18.4%).

Table (1): Powdered B. pilosa leaves - chemical formulation

Composition	Value (%)
Moisture	11.4
Protein	29.10
Fat	1.48
Ash	18.4
Crude fibers	8.08
Total carbohydrate	31.54

The chemical composition of the B. pilosa leaves powder was the same as that reported by **Deba et al.** [22].

The results in table (2) showed that dried powder of B. pilosa leaves contains carbohydrates, glycosides, sterols, triterpenoids, flavonoids, tannins, saponins and alkaloids, while Anthraquinones were absent. These consequences agreed with those of **Alvarez *et al*.** [6] and **Kviecinski *et al*.** [9] who found the occurrence of glycosides, sterols, triterpenoids, flavonoids, tannins, saponins & alkaloids in B. pilosa leaves extract.

Table (2): Phytochemical screening of B. pilosa leaves

Constituents	Results
Carbohydrates	+
Glycosides	+
Sterols	+
Triterpenoids	+
Flavonoids	+
Anthraquinones	-
Tannins	+
Saponins	+
Alkaloids	+

(+): Present, (-): Absent

Table 3 shows that the BWG% and FER of the B. pilosa alcoholic extract group were substantially higher than those of the control positive group.

This result was similar to those of **Fonnegra Gomez and Villa-Londoño,** [23] and **Sequeda-Castañeda *et al*.** [24] who reported that B. pilosa extract increased BWG % and FER.

Table (3): Effect of B. Pilosa extract on food intake, (FI), body weight gain (%) and food efficiency ratio (FER) in rats

Groups	FI (gm/ day)	BWG (%) Mean±SD	FER Mean±SD
CN	12.29± 0.27 ^{a}	55.43 ± 0.93 ^{a}	0.10 ± 0.11^{a}
CP	7.63 ± 0.25 ^{f}	33.01±1.31^{e}	0.17±0.21^{a}
B.P E 75mg	7.90 ± 0.10 ^{f}	38.21±1.23^{d}	-0.08±0.01^{b}
B.P E 150mg	8.30 ± 0.26 ^{e}	47.35 ± 0.98^{c}	-0.49 ± 0.01^{d}
B.P E 300mg	9.53 ± 0.15 ^{d}	51.0 ± 0.69 ^{b}	-0.80 ± 0.01^{e}
B.P E 450mg	10.30 ± 0.20 ^{c}	52.18 ± 1.13^{b}	-0.26 ± 1.01^{c}
RAN	11.60 ± 0.20 ^{b}	51.61 ± 0.71^{b}	-0.11 ± 0.01^{cb}
LSD	0.376	1.800	0.157

(CN) control negative, (CP) control positive, (B.P.E 75mg) Bidens Pilosa Extract 75mg, (B.P.E 150mg) Bidens Pilosa Extract 150mg, (B.P.E. 300mg) Bidens Pilosa Extract 300mg, (B.P.E 450mg) Bidens Pilosa Extract 450mg,

Bidens pilosa alcoholic extract significantly increased levels of antioxidant enzymes GPx, SOD & CAT in gastric mucosal homogenates as recorded in table (4). The antioxidant effect of Bidens pilosa extract could be attributed to the presence of its phytochemical constituents as polyacetylenes, polyacetylenic glycosides, aurons, aurons glycosides, flavonoids and flavonoid glcosides. The antioxidant effect of B. pilosa extract in this study was similar to those obtained by **Repetto and Llesuy,** [2], **Deba *et al*.** [22], **Krishnaiah *et al*.** [10] and **Kviecinski *et al*.** [9].

Table (4) Bedins pilosa extract's influence on stomach mucosal homogenate antioxidant enzyme levels: (GPx), (SOD), and (CAT)

Groups	GPx	SOD	CAT
CN	0.55 ± 0.01e	59.55 ± 1.02^{a}	4.23 ± 0.71^{dc}
CP	3.50 ± 0.10^{a}	40.49 ± 0.93^{e}	7.78 ± 0.17^{b}
B.P.E 75 mg	1.50 ± 0.10^{a}	44.47 ± 0.85^{d}	7.28± 0.30^{a}
B.P.E 150mg	1.40 ± 0.10^{b}	46.30 ± 1.68^{d}	6.23 ± 0.18^{b}
B.P.E 300mg	1.30 ± 0.10^{c}	48.59 ± 0.39^{c}	4.93 ± 0.05^{c}
B.P.E 450mg	0.84 ± 0.10^{d}	50.94 ± 0.70^{b}	3.98 ± 0.02^{d}
RAN	0.83 ± 0.05^{d}	59.29 ± 1.80^{b}	4.52 ± 0.81^{dc}
LSD	0.154	2.106	0.762

GPx= glutathione peroxidase, (U/ml), SOD= Superoxide dismutase, (U/ml),CAT= Catalase, (U/ml)
(CN) control negative, (CP) control positive, (B.P.E 75mg) Bidens Pilosa Extract 75mg, (B.P.E 150mg) Bidens Pilosa Extract 150mg, (B.P.E. 300mg) Bidens Pilosa Extract 300mg, (B.P.E 450mg) Bidens Pilosa Extract 450mg, (RAN) Ranitidine drug.

Biden pilosa alcoholic extract significantly increased levels ALT, AST and ALP as shown in table (5). These outcomes were in line with **Krishnaiah *et al*.** [10], **Bartolome *et al*.** [25]; **Monigatti *et al*.** [26].

Table (5): Changes in hepatic enzyme activity as a result of B.P.E. levels of alkaline phosphatase, alanine aminotransferase, & aspartate aminotransferase in rats

Groups	ALT	AST	ALP
CN	102.55 ± 1.19^{d}	37.36 ± 1.99^{f}	35.43 ± 0.70^{a}
CP	145.63 ± 1.10^{a}	95.46 ± 0.76^{a}	14.95 ± 0.93^{e}
B.P.E 75mg	135.53 ± 3.28^{b}	67.81 ± 1.92^{b}	14.60 ± 0.27^{e}
B.P.E 150mg	135.71 ± 1.25^{e}	55.27 ± 0.91^{c}	21.20 ± 0.90^{d}
B.P.E 300mg	127.77 ± 2.77^{c}	51.17 ± 3.39^{d}	24.49 ± 0.86^{c}
B.P.E 450mg	126.58 ± 2.71^{c}	45.50 ± 3.92^{e}	30.42 ± 0.25^{b}
RAN	103.75 ± 0.69^{d}	36.40 ± 1.11^{f}	30.27 ± 0.19^{b}
LSD	3.660	3.950	1.168

ALT= alanine aminotransferase, (U/L), AST= aspartate aminotransferase, (U/L), ALP= Alkaline phosphatase, (U/L)
(CN) control negative, (CP) control positive, (B.P.E 75mg) Bidens Pilosa Extract 75mg, (B.P.E 150mg) Bidens Pilosa Extract 150mg, (B.P.E. 300mg) Bidens Pilosa Extract 300mg, (B.P.E 450mg) Bidens Pilosa Extract 450mg, (RAN) Ranitidine drug.

Histopathological Examination

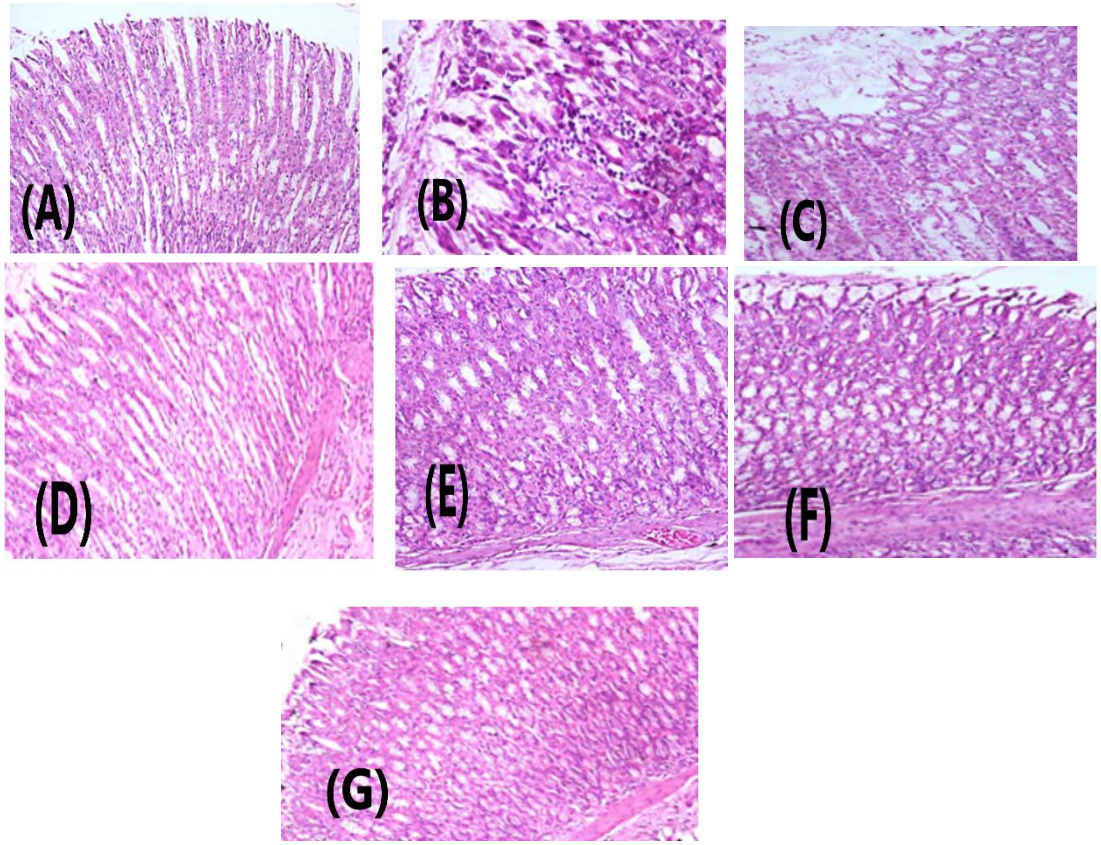

Fig (1): photomicrographs for Stomachs of rats shows, (A) Normal mucosa and submucosa in a control -ve group (H&E, X:400) . **(B)** Control +ve group showing massive necrosis in the mucosal layer, together with mononuclear cells infiltration (Blue star) and submucosal blood vessel congestion (H&E, X:400), **(C)** Group given15 mg/kg B. pilosa extract + 1ND treated showing slight regeneration in the mucosal layer with slight submucosal blood vessel congestion, together with regression of the leukocytic cells infiltration, (H&E, X:400), **(D)** Group given 75 mg/kg B. pilosa extract + 1ND treated showing slight regeneration in the mucosal layer with slight submucosal blood vessel congestion, together with regression of the leukocytic cells infiltration, (H&E, X:400), **(E)** Group given 150 mg/kg B. pilosa extract + 1ND treated showing mild regeneration in the mucosal layer with slight submucosal blood vessel congestion, together with regression of the leukocytic cells infiltration, (H&E, X:400), **(F)** Group 300 mg/kg B. pilosa extract + 1ND treated group showing moderate regeneration in the mucosal layer with moderate submucosal blood vessel congestion together with regression of the leukocytic cells infiltration, (H&E, X:400), and **(G)** Group treated by ranitidine + 1ND treated showing apparently normal stomach with healthy mucosal and submucosal layers, (H&E, X:400).

The histopathological alterations seen in the stomach of rats given indomethacin and received B. pilosa extract in the present study were like those reported by **Arlene *et al.*** [(27)] and **Bilanda *et al.*** [(28)]. These authors settled that Bidens pilosa extract induced an improvement of pathological alterations seen in the stomach of rats with gastric ulcerations.

CONCLUSION

The Bidens pilosa plant (Genus: Bidens; Family: Asteraceae) is used for therapeutic purposes. It's the gold standard for treating persistent stomach and intestine sores. The results of the current research show that B. pilosa L. extract possess gastroprotective, antioxidant hepatoprotective and ulcer healing effects. It contains many phytochemical constituents, and the presence of tannins and polyphenol compounds may explains its anti-ulcer effect.

DECLARATIONS

- **Consent for publication:** I attest that all authors have agreed to submit the work.
- **Availability of data and material:** Available
- **Competing interests:** None
- **Funding:** No fund
- **Conflicts of interest:** no conflicts of interest.

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