

Potential Protective Effects of *Plantago major* Extracts against Indomethacin-Induced Gastric Ulcer in Rats

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Abstract

Globally, gastric ulcer is one of the most serious diseases. Although, there are several antiulcer drugs, however most of them have adverse reactions. This research aims to investigate the protective effects of *Plantago major* extracts, water extract *Plantago* (WE) and ethanolic extract *Plantago* (EE) against indomethacin (IND)-induced gastric ulcer in rats. Also, to determine the active constituents and fatty acids content of *Plantago major*. Gastric ulcer was induced by a single oral dose of IND (30 mg/ kg). *Plantago* (WE) *Plantago* (EE) and Pantozol (Panto), a reference drug, were administered orally 14 days prior to IND ingestion. *Plantago major* contains high levels of total antioxidants, flavonoids, and phenols. Gas chromatography analysis showed a high percent of linoleic acid (33.79%), oleic acid (29.41%), and palmitic acid (19.25 %) in *Plantago*. Gross evaluation of gastric mucosal lesions showed that *Plantago* (WE) *Plantago* (EE) and Panto pretreatment reduced gastric lesions, diminished ulceration of surface epithelium, and maintained the normal histological structure of gastric mucosa induced by IND. Besides, significantly reduced ulcer index, total gastric acidity, gastric pH, and serum oxidative stress compared with the IND group. Pretreatment with *Plantago* (WE), *Plantago* (EE) and Panto significantly increased gastric tissue content of nitric oxide (NO) and prostaglandin E2 (PGE2). In conclusion, pretreatment with *Plantago* (WE) *Plantago* (EE) showed a better ulcer healing capacity compared favorably well with Panto results, these results could be attributed to its high content of active antioxidant constituents.

Key words: *Plantago major*, Extract, Gastric ulcer, Indomethacin, Antioxidant

Introduction

Non-Steroidal Anti-inflammatory Drugs (NSAIDs.) are extensively used for their analgesic, anti-inflammatory and antipyretic properties. However, their frequent administration is usually associated with peptic ulcer induction (**Lee et al., 2016**). Indomethacin is an important member of the NSAIDs family. It is still extensively used in joint stiffness, arthritis, and in obstetrics to delay uterine contractions. In addition it is used in the neonatal unit to facilitate patent ductus arteriosus closure. However it provokes aggressive ulcerogenic potential in both animals and humans studies (**Simon, 1993** and **Saheed et al., 2015**). Several researchers aimed to decrease damaging effects induced via indomethacin on gastrointestinal mucosa (**Soliman et al., 2016**). Indomethacin induces its gastrointestinal toxicity through several mechanisms such as an increase in gastric acid secretion; interfere with mucosal cell regeneration via inhibition of Prostaglandin E2 (PGE2) reduction of gastric nitric oxide level, and invasion of activated neutrophils, as well as induction of gastric cells apoptosis (**Matsui et al., 2011**).

There is a growing trend in the use of medicinal plant because of their medical effectiveness, low toxicity and many natural antioxidant agents derived from these plants (**Ozaslanet al., 2007**). *Plantago major*, belongs to the family Plantaginaceae, is an old medicinal plant that has been known for centuries (**Samuelsen, 2000**). The remarkable medicinal properties of *Plantago major* are due to its the high content of phenols, flavonoids and tannin especially in its leaves (**Mohamed et al., 2011**).

Plantago major L. is used as a remedy for various diseases in traditional medicine such as diseases related to the skin, respiratory organs, digestive organs, and reproduction. It has immune enhancing, wound-healing, hepatoprotective, anti-inflammatory, antigenotoxic, antioxidant and free radical scavenging effects (**Samuelsen et al., 2000; Velasco-Lezama et al., 2006** and **Nazarizadehet al., 2013**). It possesses anticancer and cytotoxic activity (**Ozaslanet al., 2009**). In addition, *Plantago major* methanolic extract displays anti ulcerogenic activity against HCL and absolute ethanol-induced gastric ulcer in rats (**Abud et al., 2012**).

The present study aimed to evaluate the potential protective action of water and ethanolic extracts of *Plantago major* against IND-induced gastric ulcer using Pantozol as a reference standard drug. Furthermore, the underlying mechanism will be explored focusing on the oxidative stress.

Materials and Methods

Plant, drugs and chemicals

Plantago major plant was obtained from Haraz for herbs and medicinal plants Company, Cairo, Egypt. Indomethacin (IND) from Hikma Pharmaceuticals PLC, Amman, Jordan, (provided as 25 mg/capsule). Pantozol® (Pantoprazole Sodium sesquihydrate, Takeda GmbH, Konstanz, Germany as 20 mg /tablet) from Pharmacy. Other chemicals and reagents were purchased from Sigma-Aldrich (St Louis, MO, USA).

Animals

Adult male rats Sprague Dawley (n=50) weighing 190-210 g were purchased from Helwan Experimental Animals Farm, Giza, Egypt.

Analysis of *Plantago major* active constituents using Gas chromatography-mass spectroscopy (GC-MS)

The assay was performed utilizing a GC-MS (Agilent Technologies 7890A) connected to a mass-specific detector (MSD, Agilent 7000). Helium was the carrier gas. The recognition of constituents was carried out by comparing their mass spectra and retention time with the library of authentic compounds (NIST and WILEY) (**Santana et al., 2013**).

Analysis of *Plantago major* antioxidant contents

Plantago major total antioxidant content were assessed by the phosphor molybdenum method (**Prieto et al., 1999**), total phenols content was evaluated by the Folin–Ciocalteu method (**Singleton et al., 1999**), and total flavonoids was assessed by Dowd method using aluminum chloride colorimetric method as adapted by (**Arvouet-Grand et al., 1994**).

Analysis of total fatty acids contents in *Plantago major*

Gas chromatography (GC) analysis of total fatty acids contents of *Plantago major* was determined by using methyl esters boron trifluoride method (AOAC, 2019). *Plantago major* powder was boiled with hydrochloric acid and then subjected to petroleum ether for fatty acid extraction. The oil is saponified with sodium hydroxide in methanol. The fatty acids are methylated with boron trifluoride in methanol, extracted with heptane, and determined on an autosampler GC with FID detector (PE Auto System XL) and Ezchrom integration system. Carrier gas (He); Ca. 25 Psi–air 450 ml/min– Hydrogen 45 ml- split 100 ml/min.

Gastric ulcer induction

Gastric ulcer was induced in all rats except for negative control group as described by **Bhattacharya et al., (2007)**. The animals were fasted for 24 h before oral administration of a single dose of IND (30 mg/kg). Different degrees of gastric mucosal injuries were detected 4 h after IND administration.

Experimental design

Adult male albino rats (n=50) weighing 190-210 g were used in this study. After one week on basal diet for acclimatization to the laboratory environment, the rats were randomized into the following 5 groups:

Group 1: (-ve control), rats received basal diet and distilled water orally.

Group 2: (IND), rats received basal diet and a single dose of IND (30 mg/kg) orally after 14 days.

Group 3: (*Plantago* WE + IND), rats received basal diet and *Plantago major* water extract (2 ml/day orally) daily for 14 days before IND.

Group 4: (*Plantago* EE + IND), rats received basal diet and *Plantago major* ethanol extract (2 ml/day orally) daily for 14 days before IND.

Group 5: (Panto + IND), rats received basal diet and Panto (20 mg/kg) daily for 14 days before IND. After feeding the groups (3, 4, 5) with the tested materials for 14 day, these rats were given orally a single dose of IND, after 4 h post IND administration, rats were sacrificed. The stomach was dissected out and cut along its greater curvature, then its content was evacuated into a centrifuge tube, diluted with distilled water and centrifuged at 12000 g for 10 min. Gastric pH and total gastric acidity were detected in the supernatant. Cleaned stomach was preserved in formalin solution at 10% and processed for macroscopic examination and histopathological examination. Blood samples were collected, serum samples were separated and kept frozen until used for biochemical determination.

Determination of gastric pH

The gastric juice was collected by a needle after tying the neck and the end of the stomach. Gastric juice (1 ml) was diluted by distilled water (1ml) in an aliquot to measure pH using pH meter (**Dashputre and Naikwade, 2011**).

Determination of total gastric acidity

Gastric fluid supernatant (1ml) was diluted in a conical flask by distilled water (1ml). Phenolphthalein indicator (2 drops) was added, then the mixture was titrated with 0.01 N NaOH till detection of a permanent pink color. The volume of 0.01N NaOH (V NaOH) consumed was recorded. The total acidity (mEq/L) was calculated (**Dashputre and Naikwade, 2011**).

Acidity = $V \text{ NaOH} \times N \times 100$

Where N is normality

Quantification of ulcer index and percentage inhibition of ulceration

Image Pro Express analyzer computer system was used to quantify the gastric ulcer index. The sum of gastric ulcer areas of all lesions for each stomach was used in the calculation of the ulcer area (mm²). The total area of mucosa and the total area of ulcers area were calculated. Then, ulcer index and percentage inhibition of ulceration were calculated according to *Meleseet al. (2011)* using the following equations:

Ulcer index (UI) = Total area of mucosal ulcers / Total mucosal area

% Inhibition of ulceration= UI (IND group) – UI (test group) / UI (IND group) × 100

Determination of serum antioxidant biomarkers

Oxidative stress biomarkers (malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT)) were determined in serum using ELISA kits obtained from My Biosource, San Diego, California, USA.

Determination of gastric protective biomarkers

Ulcer protective biomarkers nitric oxide (NO) and prostaglandin E2 (PGE2) were determined in gastric tissue using ELISA kits obtained from My Biosource, San Diego, California, USA.

Histopathological examination

The formaldehyde fixed stomach is paraffin-embedded, cut into sections, then stained with Hematoxylin-Eosin (H&E). The slides were examined microscopically.

Statistical analysis

Ulcer Inhibition was expressed in percentage. Results are reported as mean ± SD. Data were compared by one-way analysis of variance (ANOVA), followed by LSD using SPSS version 25. P < 0.05 indicates significance difference (*Armitage and Berry, 1987*).

Results

Active constituents of *Plantago major* (GC-MS)

The GC-MS analysis of *Plantago major* extract was presented in Figure (1) and Table (1). The results showed that the plant contain high percent of heptacosane (10.11%), oxiraneoctanoic acid, 3-octyl-, cis (9.34 %), phytol (8.94%), and hexadecane (8.79%), followed by squalane (6.99%), digitoxin (6.92%), nonadecane(5.82 %) and hexadecane (5.58%), and many other active constituents.

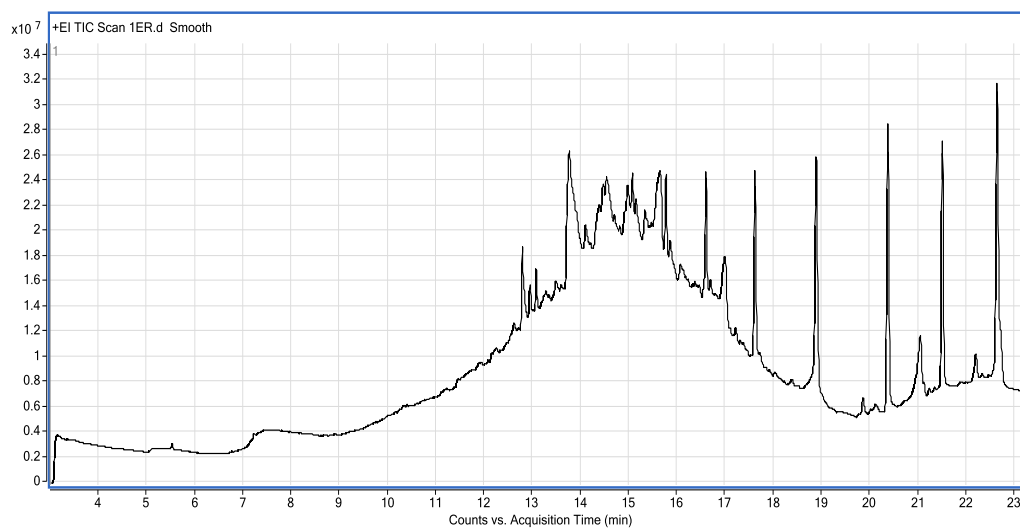


Figure (1):
Gas chromatography-mass spectroscopy (GC-MS) spectra of the *Plantago major* active constituents

Table (1)

Active constituents of *Plantago major* (gas chromatography-mass spectroscopy, GC-MS)

	Constituents	RT (min)	Concentrations (%)
1	Hyodeoxycholic acid	12.204	2.73
2	Hexadecanoic acid, 2-bromo	12.614	1.98
3	Gitoxigenin	12.827	2.26
4	Rhodopin	12.95	1.02
5	Oleic acid	13.077	0.81
6	Batilo	13.266	0.98
7	Vitexin	13.512	1.2
8	Oxiraneoctanoic acid, 3-octyl-, cis	13.778	9.34
9	Octadecanoic acid, 4-hydroxybutyl ester	14.127	1.02
10	Phytol	14.541	8.94
11	Heneicosane, 11-cyclopentyl-	14.956	0.44
12	Erucic acid	15.05	1.48
13	14-Octadecenal	15.341	1.88
14	Demecolcine	15.583	3.65
15	Geranylisovalerate	15.763	1.32
16	Heneicosane, 11-cyclopentyl	16.071	2.28
17	Octadecanoic acid, 10-oxo-, methyl ester	16.563	3.26
18	Cholic acid	16.945	2.28
19	Neronine, 4 β ,5-dihydro-	17.207	0.77
20	Hexadecane	17.605	5.58
21	Heptacosane	18.868	10.11
22	Digitoxin	19.807	6.92
23	Squalane	20.361	6.99
24	Codeine	21.021	4.01
25	Nonadecane	21.493	5.82
26	Glaucine	22.161	3.74
27	Hexadecane	22.641	8.79
28	9-Octadecenamide, (Z)	23.256	0.39
29	Non-identified compounds	> 23.26	0.01

Total antioxidants, flavonoids and phenols contents of *Plantago major*

Plantago major contain 356.63 ± 6.90 (mg/ 100 g ascorbic acid) total antioxidants, 294.13 ± 4.20 (mg/ 100 g quercetin) total flavonoids, and 9478.1 ± 20.10 (mg/ 100 g gallic acid) total phenols (Table 2).

Table (2)
Total antioxidants, flavonoids, and phenols of *Plantago major*

Antioxidant constituents	Mean \pm SE
Total antioxidants (mg/ 100 g ascorbic acid equivalent)	356.69 \pm 6.90
Total flavonoids (mg/ 100 g quercetin equivalent)	294.13 \pm 4.20
Total phenols (mg/ 100 g gallic acid equivalent)	9478.1 \pm 20.10

Values were presented as the mean of 3 replicates \pm SD.

Total fatty acids contents of *Plantago major*

Total fatty acids (FA) of *Plantago major* was presented in Figure (2) and Table (3). The *Plantago major* contains high percent of linoleic acid (33.79%), oleic acid (29.41%), palmitic acid (19.25 %), followed by lauric acid (8.53%), stearic acid (4.24%) and linolenic acid (3.43%), and low percent of gondoic acid (1,35%).

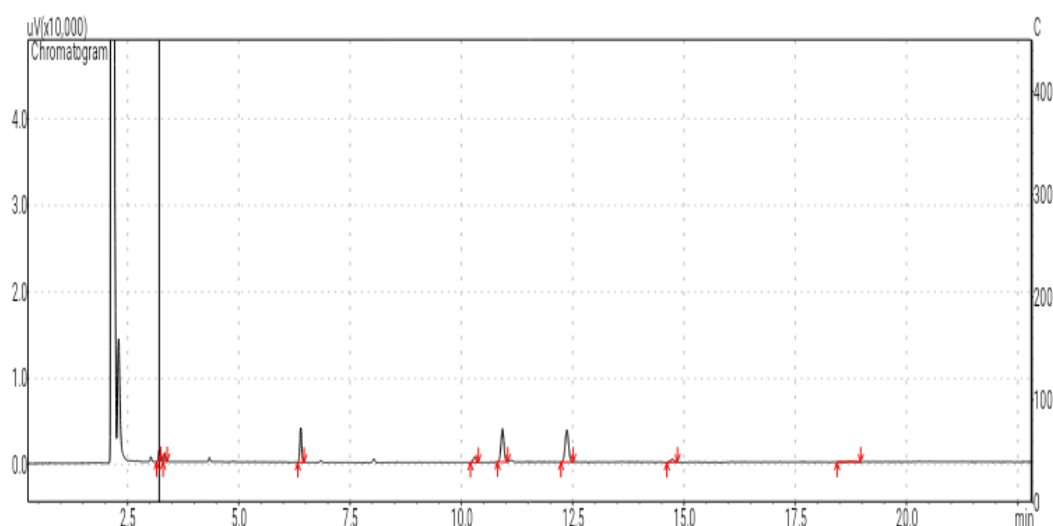


Figure (2):
Fatty acids content of *Plantago major*(gas chromatography, GC)

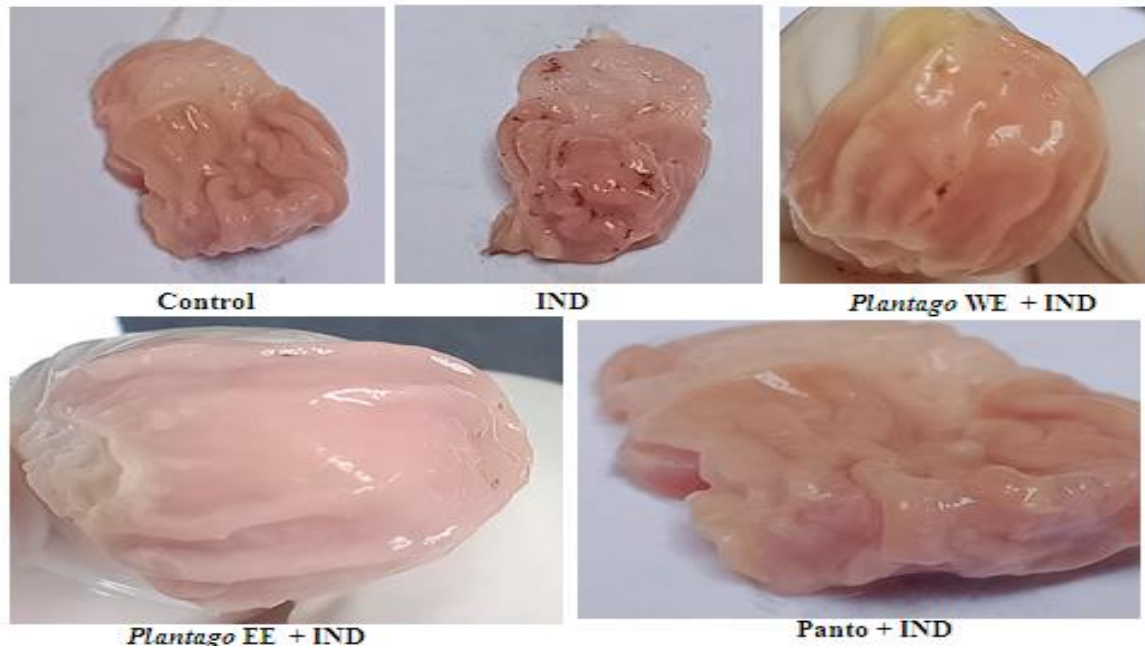
Table (3)
Fatty acids content of *Plantago major*

Fatty acids	Name	Relative distribution %
C12:0	Lauric acid	8.53
C16:0	Palmitic acid	19.25
C18:0	Stearic acid	4.24
C18:1 ω 9	Oleic acid	29.41
C18:2 ω 6	Linoleic acid	33.79
C18:3 ω 3	Linolenic acid	3.43
C20:1 ω 9	Gondoic acid	1.35

Gastric gross structure

In the IND group, the stomach showing diffuse and multiple ulcer formation with depressed centre and elevated borders. While stomach of the *Plantago* WE+IND pretreated group showing starting of ulcer healing signs. In the *Plantago* EE+IND pretreated group stomach showing advanced

ulcer healing signs. While in the Panto+IND pretreated group the stomach showing complete ulcer healing with apparently healthy intact mucosawas seen (Figure3).



Figure(3):
Effect of *Plantago major* extracts and Pantoon gastric gross structure in rats.

Stomach of the –ve control group showing intact mucosa. In IND group the stomach showing diffuse multiple ulcer formation with depressed centre and elevated borders. While stomach of *Plantago* WE+IND pretreated group showing starting of ulcer healing signs. Stomach of *Plantago* EE+IND pretreated group showing advanced ulcer healing signs. In Panto +IND pretreated group the stomach showing complete ulcer healing with apparently healthy intact mucosa.

Effect of *Plantago major* extracts on gastric mucosal lesions biomarkers

In IND group, there was a significant increase in UI compared with control group ($p < 0.001$). On the other hand, in both *Plantago* WE or EE + IND and Panto + IND groups there was a significant decrease ($p < 0.001$) in UI compared with IND group. A significant difference was found between *Plantago* WE and the other two pretreated groups in IU with 51.73%inhibition of ulceration. Besides, there was a significant difference between the Panto+IND group and the *Plantago* EE+ IND group. The percentage inhibition of ulceration accounted 55.63% and 76.49% for the *Plantago* EE+ IND and the Panto+IND groups, respectively (Table 4).

Gastric pH decreased significantly($p < 0.001$),while total gastric acidity significantly increased ($p < 0.001$) in the IND group compared with the control group. Meanwhile, gastric pH increased significantly concurrent with a significant decrease in total gastric acidity in *Plantago* WE + IND, *Plantago* EE +IND, and Panto + IND groups compared with the IND group ($p < 0.001$).In total gastric acidity, a significant difference was found between water *Plantago* extract and the other two pretreated groups. Besides, there was a significant difference between the Panto+IND group and the *Plantago* EE+ IND group (Table 4).

Table(4)

Effect of *Plantago major* extracts and Panto on ulcer index (UI), percentage inhibition of ulceration, gastric pH, and total gastric acidity in IND-induced ulceration in rats.

Experimental groups	UI (mm ²)	Inhibition of Ulceration (%)	Gastric pH	T. gastric acidity
Control	-	-	5.05 ±0.24	56.35± 2.51
IND	10.21± 0.87 ^a	-	2.23 ± 0.23 ^a	111.50 ± 6.11 ^a
Plantago WE + IND	5.03 ± 0.33 ^b	51.73	4.12 ± 0.39 ^b	81.05 ± 4.41 ^b
Plantago EE + IND	4.53 ± 0.41 ^{b,c}	55.63	4.06 ± 0.24 ^b	73.47 ± 3.86 ^{b,c}
Panto + IND	2.40 ± 0.35 ^{b,c,d}	76.49	3.91± 0.22 ^b	64.41 ± 3.62 ^{b,c,d}

Data are represented as mean ± SD (n = 10).

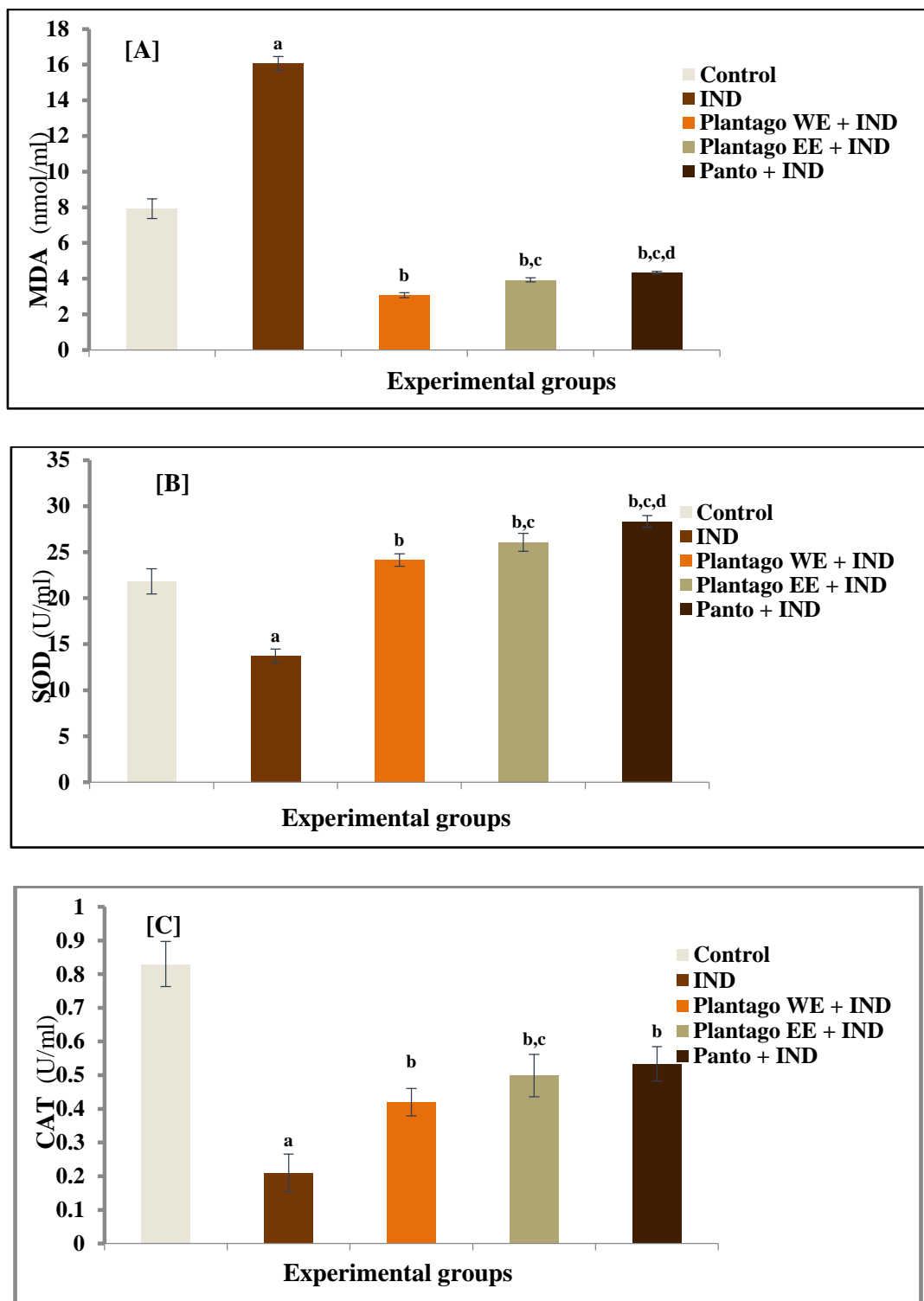
^a Significant versus control, ^b significant versus IND, ^c significant versus *Plantago* WE + IND, and ^d significant versus *Plantago* EE + IND (p≤ 0.05).

Effect of *Plantago major* extracts on serum oxidative stress biomarkers

In IND group, there was a significant increase in the serum MDA level with significant decrease in the serum SOD and CAT levels compared with control group (p< 0.001). On the other hand, in *Plantago* WE+ IND, *Plantago* EE + IND and Panto + IND pretreated groups there was a significant decrease (p<0.001) in MDA concurrent with significant increases in the SOD and CAT levels compared with the IND group. A significant difference was found between *Plantago* WE+ IND group and the *Plantago* EE +IND in all tested oxidative stress biomarkers. Besides, there was significant differences between the Panto+IND group and the *Plantago* extracts pretested group in the serum levels of MDA and SOD (Figure 4).

Effect of *Plantago major* extracts on gastric protective biomarkers

In IND group, there were significant decreases in the gastric NO and PGE2 levels compared with the control group (p< 0.001). On the other hand, in both *Plantago* WE or EE + IND and Panto + IND groups there were significant increases (p<0.001) in gastric NO and PGE2 level compared with the IND group. A significant difference was found between water *Plantago* extract+ IND group and both the *Plantago* EE+IND and Panto+IND groups in gastric NO content (Table 5).



Figure(4):

Effect of *Plantago major* extracts and Panto on serum antioxidant malondialdehyde (MDA) [A], superoxide dismutase (SOD) [B], and catalase (CAT) [C] levels in IND-induced ulceration in rats. Data are represented as mean \pm SD (n = 10).

^a Significant versus control, ^b significant versus IND, ^c significant versus *Plantago* WE + IND, and ^d significant versus *Plantago* EE + IND (p \leq 0.05).

Table (5)
Effect of *Plantago major* extracts and Panto on gastric nitric oxide (NO) and prostaglandin E2 (PGE2) concentrations in IND-induced ulceration in rats

Experimental groups	NO (µmol/g)	PGE2(ng/g)
Control	7.02 ± 0.35	120.62 ± 4.77
IND	2.29 ± 0.37 ^a	85.87 ± 4.36 ^a
<i>Plantago</i> WE + IND	4.91 ± 0.36 ^b	115.36 ± 5.35 ^b
<i>Plantago</i> EE + IND	5.81 ± 0.53 ^{b,c}	117.63 ± 4.11 ^b
Panto+ IND	6.25 ± 0.74 ^{b,c}	119.06 ± 3.43 ^b

Data are represented as mean ± SD (n = 10).

^a Significant versus control, ^b significant versus IND, ^c significant versus *Plantago* WE + IND, and ^d significant versus *Plantago* EE + IND (p ≤ 0.05).

Effect of *Plantago major* extracts on the gastric tissue histopathological changes

The stomach of the (-ve) control group shows normal stomach layers; normal lamina epithelialis, lamina propria, submucosa, and muscularis mucosa (Figure5.A). In IND group, the stomach showing ulcerative gastritis, complete destruction of all stomach layers with mononuclear cells infiltration and congested blood vessels (Figure5.B). While The stomach from the *Plantago* WE + IND group showing early stages of ulcer healing; the immature granulation tissue formation, mononuclear cells infiltration, and dilated blood vessels (Figure5.C). Stomach from the *Plantago* EE + IND group showing advanced stages of ulcer healing; the mature granulation tissue formation and the mononuclear cells infiltration (Figure5.D). Stomach from the Panto+ IND group showing complete ulcer healing; the intact stomach layers; mucosa, submucosa, and musculo (Figure5.E).

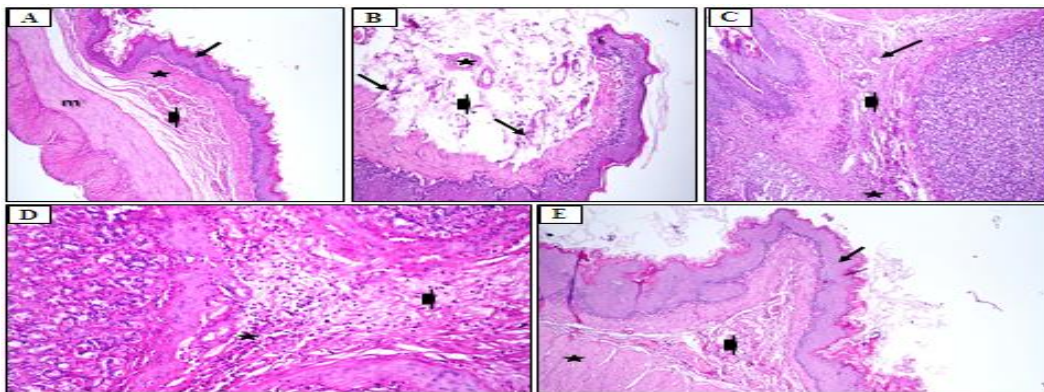


Figure (5):
Effect of *Plantago major* extracts and Panton histopathological alteration on gastric tissue in IND-induced ulceration in rats (H&E X 200).

The stomach from control group showing normal stomach layers; note the normal lamina epithelialis (arrow), lamina propria (*), submucosa (arrow head), and muscularis mucosa (m) (Fig. A). In IND group showing ulcerative gastritis; note the complete destruction of all stomach layers (arrow head) with mononuclear cells infiltration (arrows) and congested blood vessels (*) (Fig. B). Stomach from the *Plantago* WE + IND group shows early stages of ulcer healing; note the granulation tissue formation (arrow head), mononuclear cells infiltration (*), and dilated blood vessels (arrow) (Fig. C).

Stomach from the *Plantago* EE + IND group shows advanced stages of ulcer healing; note the mature granulation tissue formation (arrow head) and the mononuclear cells infiltration (*) (Fig. D). Stomach from the Panto + IND group shows complete ulcer healing; note the intact stomach layers; mucosa (arrow), submucosa (arrow head), and muscosa (*) (Fig. E).

Discussion

Gastric ulcer is the most prevalent gastrointestinal disorders and the complications worldwide (**Chow and Sung, 2009**). Although conventional treatments are effective, both clinical and experimental studies have demonstrated that traditional herbal medicines exhibit therapeutic benefit for gastric ulcer (**Biet al., 2014**). The phyto constituents found in *Plantago major* as flavonoids, tannins, steroids, triterpenoids, phenolic acids, and alkaloids have antioxidant properties (**Nayaket al., 2011** and **Nyi and Dytha, 2019**). These components are known to protect the gastric mucous membranes (**Kobeasyet al., 2013**). The present study evaluated the gastroprotective effects of *Plantago major* water and ethanol extracts against IND-induced ulcers in rats. Besides, comparing its effects with Panto as reference drug.

In the present study the total antioxidants, total flavonoids and total phenols content of *Plantago major* were major to that obtained by **Kobeasyet al., (2011)** that the ethanolic extract of *Plantago major* leaves, had the highest total phenols, flavonoid and tannin contents which explains the highest antioxidant activity of ethanol extract of *Plantago major* leaves. **Jyotiet al., (2017)** reported that *Plantago major* extracts have antioxidant compounds, which is used to prevent diseases related to oxidative processes. While, different results were found by **Karimaet al., (2015)** who reported that the total phenols and total flavonoids content of different extracts from *Plantago major* leaves were (petroleum ether, ethyl acetate and aqueous fractions) were low .

Plantago major contained high percent of linoleic acid, oleic acid, palmitic acid .These finding agreed with those obtained by **Liu et al., (2002)** who reported that the major fatty acids of *Plantago major* leaves were linoleic and palmitic acid. **Kobeasyet al.,(2011)** reported that *Plantago* leaves has a high percentage of linolenic acid. **Manjari and Das, (2000) and Manjari and Das, (2000)** reported that increased dietary intake of polyunsaturated fatty acids (PUFAs) is known to be associated with a decrease in the incidence of peptic ulcer disease possibly due to increase in the synthesis of prostaglandins. Diets or supplements high in linolenic acid (C18:3) and linoleic acid (C18:2), polyunsaturated fatty acids (PUFAs) are required for PGE2 synthesis(**Kirkupet al., 2010**), the PGE2 exerts a potent protective activity *in vitro* which is independent on neural, vascular and hormonal factors (**Brzozowskiet al., 2005**).

In the present study, there was a significant increase in UI in the IND group compared with the control group. On the other hand, in both *Plantago* WE or EE+IND and Panto + IND groups there was significant decrease in UI compared with IND group. A significant difference was found between water *Plantago* extract and the other two pretreated groups with 51.73% inhibition of ulceration. Besides, there was a significant difference between the Panto+IND group and the *Plantago* EE+ IND group. Our results agree with **Atta and El-Sooud, 2004; Kim et al., 2011** and **Moramet al., (2016)**. Increasing in ulcer index in the ulcerated rats might be ascribed to either inhibition of prostaglandin synthesis or formation of free radicals (**Atta et al., 2005** and **Chadanet al., 2013**).The gastroprotective effect of *Plantago major* could be attributed to its content of variable amounts of triterpens, flavonoids, tannins, and polyunsaturated fatty acid like linoleic acid (**Atta and Mounair, 2004** and **Rakeshet al., 2011**).

Triterpens are the active constituents that have been claimed to be effective as an anti-ulcer agent because it protects the mucosa against acid effects (*De Pasquale et al., 1995*). In addition, tannins is an astringent which forms a protective layer of Pellicle over the lining in the site of ulcer thereby to prevent the absorption of toxic substances and to resist the attack of the proteolytic enzymes (*Nwaforet al., 1996*).

The pH gives an indicator of gastric secretions acidity. Low pH value is a sign of low concentration of hydrogen ions in gastric juice. This was associated with ulcer disease and gastric harm in animal models (*Lullmann et al., 2000*). In the present study, gastric pH increased significantly concurrent with significant decrease in total gastric acidity in both *Plantago* WE or EE extracts + IND, and Panto + IND groups compared with the IND group. In total gastric acidity, a significant difference was found between water *Plantago* extract and the other two pretreated groups. Besides, there was a significant difference between the Panto +IND group and the *Plantago* EE+ IND group. several studies showed that indomethacin caused alterations in gastric secretions of rats. Low pH value has been linked to causing ulcers and destroying the stomach in animals (*Yesildaet al., 1993; Beck et al., 2000; Biplabet al., 2011* and *Muhammed et al., 2012*). This lesion occurs through internal defeats such as pepsin and oxidative stress and external factors such as drugs and chemicals that damage the gastric mucosal epithelium (*Lullmann et al. 2000* and *Gazwi and Mahmoud, 2018*).

Indomethacin stimulates the metabolites of reactive oxygen in animal experiments, which may participate to mucosal damage and devastate cellular antioxidant enzymes that act as the first cellular defense line against oxidative damage (*El-Missiryet al., 2001 and Chattopadhyay et al., 2006*). In this study there was a significant increase in the serum MDA level with significant decreases in the serum SOD and CAT levels in the IND group compared with the control group. On the other hand, in both *Plantago* WE or EE + IND and Panto + IND pretreated groups there was a significant decrease in MDA concurrent with significant increases in the SOD and CAT levels compared with IND group. These results agree with (*Kim et al., 2011; Allam and EL-Gohary, 2017* and *Nwaonukuruet al., 2018*). *Plantago* WE or EE + IND showed a noticeable suppression of oxidative damage because of its radical scavenging activity, it conveyed MDA level nearer to ordinary levels. This could be explained by the activated components in *Plantago* extract that contain flavonoids and terpenoids which play vital role as a scavenger of free radicals and perform powerful antioxidant function (*Ali et al., 2013*).

In IND group, there was significant decrease in the gastric NO and PGE2 levels compared with the control group. On the other hand, in both *Plantago* WE or EE + IND and Panto + IND groups there were significant increase in gastric NO and PGE2 levels compared with IND group. This result agree with *Lanaset al., (2000)* and *Cadirciet al., (2007)*. The decrease in NO biosynthesis may be due to diminished activity of NO synthase (NOS) (*Tripp and Tepperman, 1995*). The indomethacin causes reduction of the activity of endothelial nitric oxide synthase (eNOS) and thus reduce the level of NO (*Slomiany et al., 1999*). All pretreated groups showed significant increase in NO levels compared to the IND group. The potential mechanism for increasing the NO level in *Plantago* WE or EE pretreated groups could be attributed to its flavonoids content which have potent antioxidant and gastro protective properties (*Matsuda et al., 2003*).

Histological examination of the stomachs demonstrated that the stomach from the IND group showing ulcerative gastritis, complete destruction of all stomach layers with mononuclear cells infiltration and congested blood vessels. These results agree with several studies where the indomethacin caused marked damage to the gastric mucosa such as focal coagulative necrosis, leukocytic infiltration, and blood vessels congestion (*EL-Moselhyet al., 2009; El-Demerdash et al.,*

2010 and *Shahin et al., 2018*). These results could be attributed to a significant increase in lipid peroxidation and generation of oxygen free radicals (*Kim et al., 2011*). While the recovery of gastric mucosa of *Plantago* WE, EE + IND and Panto+ IND presumably relies upon vaso ligating action. These action increments mucosal blood flow and promote angiogenesis, a key factor in ulcer recuperating (*Kontureket al., 1993*).

In conclusion, our results showed that water and ethanol extracts of *Plantago major* showed gastroprotective effects against IND-induced gastric ulcer. These impacts may be due to the presence of flavonoids, tannins, steroids, triterpenoids, phenolic acids, alkaloids and polyunsaturated fatty acids in *Plantago* extracts which induce inhibition in lipid peroxidation and enhancement in enzymatic antioxidant defenses.

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التأثير الوقائي لمستخلص لسان الحمل على قرحة المعدة المُحدثه بواسطة الإندوميثاسين في الفئران

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قرحة المعدة تعتبر من أخطر الأمراض عالمياً. بالرغم من وجود العديد من الأدوية المستخدمة لعلاج القرحة، إلا أن أغلبها له بعض التأثيرات السلبية. يهدف هذه البحث الى دراسة التأثير الوقائي للمستخلص المائي والإيثانولي لنبات لسان الحمل ضد قرحة المعدة التي يسببها الإندوميثاسين في الفئران. وأيضاً تقييم محتوى لسان الحمل من المركبات الفعالة والأحماض الدهنية. تم احداث القرحة بواسطة جرعه واحده فمويه من الإندوميثاسين (30 ملجم/كجم). تم اعطاء الفئران بالفم المُستخلص المائي والإيثانولي و عقار البننزول (البننتو)، كعلاج مرجعي، لمدة 14 يوم لكل مجموعته على التوالي قبل إعطاء الإندوميثاسين. أظهرت النتائج أحتواء لسان الحمل على معدلات عاليه من مضادات الأكسده الكليه، الفلافونيدات والفينولات. وأظهرت نتائج جهاز التحليل الكروماتوجرافي احتواء مستخلص لسان الحمل على نسبة عالية من حمض اللينوليك (33,79%)، حمض الأوليك (29,41%)، وحمض البالمتيك (19,25%). أظهر فحص الغشاء المخاطي المعدي ان المُستخلص المائي والإيثانولي لنبات لسان الحمل المائي، و عقار البننتو أحدث تأثيراً واضحاً في أنخفاض الاضرار الناجمة عن الإندوميثاسين والتقرحات السطحية، مع المحافظة علي التركيب النسيجي الطبيعي للغشاء المخاطي المعدي. بالإضافة الي حدوث إنخفاض ذي دلالة إحصائية في مؤشر القرحة، الحموضة الكلبيه في المعدة، الرقم الهيدروجيني، والاجهاد التأكسدي في مصل الدم مقارنة بالمجموعة المصابة بالقرحة. وقد أدت المعالجة المسبقة باستخدام المُستخلص المائي والإيثانولي لنبات لسان الحمل المائي، و عقار البننتو الي حدوث إرتفاعاً ذي دلالة إحصائية في محتوى نسيج المعدة من أكسيد النيتريك والبروستاجلاندين. بناء علي النتائج المتحصل عليها، أظهرت المعالجة المسبقة باستخدام المُستخلص المائي والإيثانولي لنبات لسان الحمل المائي قدرة في الحماية من القرحة المعدية عند مقارنتها بنتائج عقار البننتو ، ويمكن أن ترجع هذه النتائج الي المحتوى العالي لنبات لسان الحمل من المواد الفعالة النشطة التي لها خصائص مضاده للأكسده.