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Gastroprotective actions of hydroethanolic extract of *Parapholisincurva*on aspirin and ethanol induced gastric ulcer in rats via histological, histochemical, immunohistochemical and biochemical assessments

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Abstract

Recently, people choose alternative and complementary therapies, especially remedies from herbs, because they are less costly and have less side effects. Currently, the gastroprotective properties of a hydro-ethanolic extract of *ParapholisIncurva* (PI) were evaluated against the gastric ulcer-induced rats sing the two ulceration methods by aspirin (AS) and ethanol (EtOH), separately, based upon the biochemical, histochemical, and immunohistochemical assessments. Oxidative stress markers in AS and EtOH treated groups showed an increase in MDA and decrease in GSH levels which all were markedly improved in groups that were pretreated with PI extract comparing with the ranitidine as a standard anti-ulcer drug. AS and EtOH significantly altered the antioxidant status. The macroscopic appearance of the stomach of experimental rats showed the aspirin treated group in the glandular stomach, the most evident finding was linear hemorrhagic lesions and patchy pale lesions accompanied hemorrhagic lesions in the glandular stomach. Also, the light microscopic examination revealed many histological alterations such as loss of surface epithelial cells, numerous necrosis of gastric mucosa, submucosal vacuolation of the glandular portion, degenerative changes in the gastric gland and congesting blood vessels. A notable decrease in the Periodic Acid-Schiff (PAS) reaction accompanied by a notable rise in the area % of collagen fibers. When AS and ethanol were administered to the groups, the immunohistochemistry stain showed that caspase-3 and COX2 expression was dramatically up regulated while COX1 decreased. A marked protection in the glandular architecture of the gastric mucosa was observed in the PI extract treated groups within a mucosal surface almost similar to the control. PAS reaction revealed partly preserved surface epithelium and glandular epithelium with a marked protection in PAS-positive surface mucous cells. A notable decrease in the area % of collagen fibers.The immunohistochemistry stain showed that caspase-3 and COX2 expression was downregulated while and COX1 increased. The total phenolic and flavonoid content assays revealed that this extract is very rich with polyphenolic, including phenolic and flavonoid, components. The gastroprotective action of this plant might be attributed to this significant antioxidant and anti-inflammatory components. These results revealed that the PI extract might be helpful in preventing stomach ulcers induced by EtOH or AS.

Keywords: *Parapholisincurva*, Ethanol-gastric ulcer, Aspirin, Histology, immunohistochemical, Phenolic compounds.

1. Introduction

The most innocuous lesions to the stomach are gastric ulcers, which are nevertheless a common clinical issue in our setting. mostly affecting people of all ages and genders, and having a big global influence on patient quality of life and health economics **[1].** Because of an imbalance between aggressive and defensive elements, the stomach mucosal defense mechanisms are disrupted, leading to bleeding, perforation, and erosion of the gastric mucosa, which exacerbates gastric ulcers **[2]**. Among the most commonly given antipyretic, anti-inflammatory, and analgesic is aspirin. NSAID is also applied in the prophylaxis of thrombotic disorders of the heart. Despite its therapeutic

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benefits, its ability to cause stomach ulcers in humans and animals has limited its use in a range of clinical disorders **[3,4]**. The development of aspirin-induced gastric ulcers encompasses a suppression of cyclooxygenase enzymes, which blocks the synthesis of certain prostaglandins (PGs). Additionally, the alteration of microvascular structures weakens the defensive gastric mucosal barrier, reduces mucosal blood flow, and damages epithelial tissue. Proinflammatory mediators and free radicals are released, leukocyte infiltration increases, and antioxidant enzymes that predispose to ulcers are decreased**[5]**.

Overindulgence in alcohol can cause or worsen a number of diseases. It is thought to be particularly significant for inflammatory and ulcerative lesions of the stomach mucosa **[6,7]**. The pathophysiology of this lesion is still not well known, though. Thus, it would be reasonable to assume that long-term EtOH use that causes stomach mucosal ulcers will affect the kinetic balance between cell death and proliferation. According to several observations, EtOH-induced stomach cell proliferation may be linked to alterations in oxidative stress, lipid peroxidation, and epidermal growth factor receptors **[8]**.

In recent years, people favor complementary and alternative medicine, especially herbal remedies, as they are less costly and have less side effects. The inherent antioxidant activity of phytochemicals in medicinal plants has attracted interest because it lessens oxidative damage caused by free radicals. It is advantageous to consume edible plant parts high in antioxidant and total phenolic compounds to prevent oxidative damage **[9].** There have been reports of the use of medicinal plants to prevent induced gastric ulcers in laboratory animals and the treatment of peptic ulcers with plant products used in folk medicine **[10]**. Both in developing and wealthy nations, there is an increasing demand for medicinal plants since they are easy to get, reasonably priced, non-narcotic, and have fewer adverse effects **[11]**.

According to Vinothapooshan and Sundar[12], there have been reports of anti-ulcerogenic activity and good stomach protection associated with phenolic compounds and flavonoids. Native to Europe, Asia, and northern Africa is the grass species *P. incurve* (family: Poaceae). Because of its high flavonoid and phenolic contents, this plant has been shown to have ameliorative effects on pathological changes in the thyroid gland caused by sodium fluoride in rats **[13]**.

The objectives of the current work are the evaluation of protection capabilities of the hydroethanolic extract of *P. incurve* against the stomach ulceration by aspirin (AS) and ethanol (EtOH) in rats via the histological, histochemical, and immunohistochemical assessments.

2. Material & methods

2.1. Drugs and chemicals

Aspirin (AS), and ranitidine (RA), were acquired from SEDICO Pharmaceutical Company, Giza, Egypt, and Bayer BitterfeldGmbh, Germany, respectively. Carboxymethyl cellulose (CMC, 1%) was used to dissolve aspirin. RA was provided as a white powder and dissolved in distilled water. The EtOH (99.9%) that used in gastric induction was purchased from Chong Yu Hi-Tech Chemicals (China). COX-1, COX-2, and caspase 3 rabbit polyclonal antibodies were purchased from Thermo Fisher Scientific (USA). Kits for the determination of liver, kidney function and antioxidant parameters were purchased from Biodiagnostics Co. (Giza, Egypt). All chemicals were of analytical grade.

2.2. Collection of the plant materials

During *P. incurva*'s flowering season in March-April 2021, the aerial parts were gathered from Kafr-Saad, Damitta, Egypt. Prof. Ahmed M. Abd-El-Gawad, a taxonomy professor, Mansoura University, authenticated the plant. At the Mansoura University Herbarium in Egypt, a Voucher sample specimen (DA-X317-015-M1921) was deposited.

2.3. Extraction and chemical analysis process

After that, the *P. incurve* aerial parts were allowed to air dry completely and then crashed to powder via the sanitized plant grinder. The 850 g of air-dried powdered plant were extracted with 70 % EtOH in ratio, filtered, and vacuum-dried at 40 C to yield 36.6 g of dark black gummy extract.

2.4. Assaying of total flavonoid (TFC) and phenolic (TFC) contents

According to previously published descriptions, the contents of total flavonoid (TFC) and total phenolic (TFC) were quantified of the *P. incurve***[14].**

2.5. Experimental animals

One hundred and twenty adult male albino rats (weigh: 150-200 g; Age: 12-16 weeks), were employed in this investigation for overall experiments including acute toxicity (50 rats) and gastroprotection evaluations (70 rats). Rats were obtained from the National Research Center's Lab Animal House. The rats were acclimated to laboratory surroundings for two weeks before the start of experiment. Throughout the whole testing time, the animals were housed in an air-conditioned room with a temperature of 24±2 °C and a relative humidity of 55±5% with alternation of 12 hours of light and dark cycles. Water and food were freely accessible to rats.

2.6. Acute toxicity assessment

To establish a safe dosage for the extract, an acute toxicity test was conducted on the plant extract. The LD_{50} was determined using the guidelines of the OECD 4235 protocol **[15].** Five groups (10 rats, each) of fifty male rats were chosen randomly. Just distilled water was given to one group, which is the control and four groups, with 10 rats per group, and given varying doses of the extract orally. Prior to receiving the extract, rats were kept fast for a whole day to keep the stomach empty. A further 3-4 hs were spent without food after administration. Oral dosages of 400, 800, 1600, and 3200 mg/kg body weight of the extract suspended in distilled water were given. Rats were then subjected to monitoring any signs of clinical or toxicological manifestations. If any deaths occurred, they were noted over. For a period of two weeks, the animals were kept under surveillance. Throughout the trial, the animal models' neurological and autonomic behaviors were observed such as diarrheal, weakness, tremors, seizures, or lack of regulated movement **[16]**. Upon completion of the experiment, the rats that had fasted for the previous night were sedated by inhalation, and blood samples were taken from the retro-orbital sinuses using capillary tubes. The serum was separated and kept in a freezer at a temperature of -80°C until additional analyses were performed to assess the impact of *P. incurva* extract on renal and liver parameters. These included total protein, albumin, globulin, TB, CB, AP, GGT, AST, and ALT as liver biochemical parameters and sodium, potassium, chloride, CO_2, urea and Creatinine for kidney biochemical parameters in the administered rats. The goal was to observe any differences in these parameters when compared to the control group of normal rats. Standard procedures were used to determine the serum biochemical parameters **[17].**

2.7. Experimental design

Ten rats in each group of seven treated groups were randomly assigned. Prior to the experiment, the animals were fasted for 24 hs to keep the stomach empty, water was permitted **[18].Group 1** (control) received an oral dose of 0.85 % saline solution. **Group 2 (AS group): g**astric ulcer induced by aspirin400 mg/kg b.wt taken orally as a single dose model according to **[19]**. **Group 3 (AS+PI group):** oral *P. incurve* extract (400 mg/kg mg b.wt) was administered to the rats two hours before AS treatment. **Group 4 (AS+Rantidine group):** oral ranitidine (50 mg/kg b.wt) was administered to the rats two hours before to the AS treatment according to **[20]**.**Group 5 (EtOH group):** As a single dosage model to cause stomach ulcers, the rats were given 1 ml absolute alcohol/100g b.wt orally **[4]**. **Group 6 (EtOH+PI group):** oral *P. incurve* extract (400 mg/kg mg b.wt) was administered to the rats two hours before EtOH treatment. **Group 7 (EtOH+Rantidine group):** oral ranitidine (50 mg/kg b.wt) was administered to the rats two hours before to the EtOH treatment **[20]**. After 60 minutes ulcer induction, animals were put to sleep in a desiccator using a cotton ball soaked in diethyl ether for two to five minutes. Each anaesthetized rat's stomach was removed right away, opened along its larger curvature, and carefully cleaned in 10% formalin to eliminate any stomach contents before being spread out on a piece of cork on glass plates with the mucosal surface facing up for macroscopic inspection.

2.8. Gross ulcer index and damage area calculations

A digital camera was used to take pictures of the stomach mucosal sides in order to assess the extent of overall mucosal damage according to **[21]**. The gross ulcer index of all the lesions in each stomach was added up to determine the overall damage score for that stomach. Furthermore, the computer imaging program measured the damage area. The UI was calculated using the following formula **[22]**.

$$UI\% = [(UA \text{ of ulcer induced group} - UA \text{ of treated}) / UA \text{ of ulcer induced group}] \times 100$$

Where UI=Ulcer Inhibition, and UA=Ulcer Area

2.9. Histological Examination

2.9.1. Light Microscopic Examination

Sections of the stomachs were cut using a rotatory microtome to a thickness of 5 µm, preserved for 48 hours in 10% formalin, gradually dried in ethyl alcohol, cleaned in xylene, infiltrated, and embedded in melted paraffin wax. The general histological structure of paraffin sections (5 µm) was examined using Hematoxylin and Eosin (H&E) stain, collagen fibers were highlighted with Mallory's Trichrome stain, and mucous materials were highlighted with Periodic Acid-Schiff (PAS) staining technique **[23].**

2.9.2. Immunohistochemical analysis

In accordance with **Okkay et al. [24]**, caspase 3 was stained immunohistochemically. The sections in series dewaxed, hydration, and immersion in an antigen retrieval solution (pH 8 EDTA). After applying protein block and 0.3% hydrogen peroxide, they were incubated with a 1:100 dilution of the caspase 3 primary antibody (RandD Systems Inc., Minneapolis, Minn., USA). After three PBS rinses, the slides were incubated for 30 minutes at room temperature with anti-mouse IgG secondary antibodies (EnVision + System HRP; Dako). They were then visualized using commercial di-aminobenzidine kits (Liquid DAB+Substrate Chromogen System; Dako), and counterstained with Mayer's haematoxylin. Normal mouse serum was used in place of the main antibody as a negative control.

The stomach slices were stained with cox-1 and cox-2 primary antibodies using immunohistochemical (IHC) analysis (Thermo

Fisher Scientific, Fremont, California, USA). As previously mentioned, the Ventana Bench Mark system was used to automatically complete the IHC steps **[25].** Sections were examined with light microscope (Leica:DM-1000).

2.10. Biochemical analysis

2.10.1. Determination of malondialdehyde (MDA) content

The methods of **Yoshioka et al.[26]**was applied. After being washed with sodium chloride solution (0.9%), 100 mg of the stomach was homogenized in 10 L of ice-cold potassium chloride solution (1.15%) using a polytron homogenizer (PT3100). 0.5 ml of the homogenate was mixed with 3 ml of trichloroacetic acid (TCA, 0.5%) and 1 ml of tertiary butyl alcohol (TBA, 0.6%), stirred, and boiled for 45 minutes in a boiling water bath. After cooling, 4 ml of n-butanol was added and quickly swirled. The n-butanol layer was separated using centrifugation for 15 minutes at 3000 rpm. Using a double beam spectrophotometer (Shimadzu, UV-PC160, Japan) against a blank that had water in place of the sample, the absorbance of the pink product was determined at 535 nm.

2.10.2. Determination of Glutathione (GSH) levels

Using the **Ellman** technique **[27]**, the amount of GSH in the stomach homogenate (GSH nmol/g tissue) was determined. After an aliquot from the generated tissue homogenate was allowed to react with 5,5-ditiobis-2- nitrobenzoic acid (DTNB), the absorbance at 412 nm was measured using a spectrophotometer.

2.11. Statistical analysis

All statistical data were analyzed using SPSS Inc.'s statistical program for Social Science, version 20 (Chicago, Illinois, USA). Group mean variations were statistically significant according to one-way analysis of variance. All results were given as mean ± Standard Deviation (mean ± SD) and a probability of $P < 0.05$ was considered statistically significant **[28]**.

3. Results and discussion

3.1. Estimation of TFC and TPC contents and main chemical phenolic compounds

The total phenolic (TP) and flavonoid (TF) contents of *P. incurve* hydroethanolic extract were estimated and presented in (**Table 1**). The results exhibited the high phenolic and flavonoid contents with respective values of 139.23 mg gallic acid equivalent/g dry extract wt and 73.01 mg quercetin equivalent /g dry extract wt**.** The findings exhibited that this plant is very rich with the polyphenolic components including the phenolic compounds and flavonoids.

Table 1: Total phenolic (TP) and flavonoid (TF) contents of *P. incurve* hydroethanolic extract.

TPC (mg gallic acid equivalent/g dry extract wt)	139.23 ± 17.64
TFC (quercetin equivalent mg/g dry extract wt)	73.01 ± 3.65

± standard deviation (n=3)

3.2. Acute Toxicity

After giving albino rats a single dose (400,800,1600, or 3200 mg kg-1 of *P. incurve* extract), there were no deaths for the course of the 14-day trial period. Even at the maximum dose (3200 mg kg^{-1}), all of the animals were able to survive and showed no signs of physical toxicity, as shown by their normal breathing patterns and the lack of tremors, convulsions, diarrheal, salivation, or paralysis in the treated animals. There were also no differences in body weight **(Table 2)**. Clinical findings and blood biochemistry data for the kidney and liver did not reveal any appreciable variations between the treated and control groups (**Tables 3&4**). These results were attributed directly with the safety of the *P. incurve* extract that clearly appeared via the stability of the body weights of the rats.

Table 2: The mean initial and final body weight of the rats of different toxicity assays

Body weight (g± SD)	*P. incurva* extract (mg/kg)			
	400	800	1600	3200
Starting b. wt.	161.8± 7.1	155.8±5.1	175.3± 5.3	162± 8.5
Final b. wt.	185± 5.8 ▲	184.3± 4.8 ▲	188.7±7.5▲	181.8± 8.6▲

SD: standard deviation (n=10 rats); ▲ A significant increase compared with control group; The data were treated within One-way ANOVA analysis

Table 3: Effects of *P. incurva*hydroalcoholic extract on the kidney parameters in male rats

Group		Parameter (mmol/L)					
		Sodium	Potassium	Chloride	CO_2	Urea	Creatinine
Normal group		134.35±0.84	5.26±0.04	95.67±0.42	22.15±0.21	6.04±0.22	25.81±2.76
*P. incurva*extract (mg/kg)	400	135.62±0.71	4.98±0.16	96.13±1.45	22.07±0.40	6.17±0.20	25.56±1.57
	800	131.67±0.70	5.56±0.34	95.17±1.32	21.08±0.84	6.54±0.17	26.66±2.50
	1600	133.64±0.84	5.76±0.50	96.15±0.15	22.03±0.32	5.98±0.24	25.50±1.53
	3200	132.61±0.32	4.88±0.75	95.14±1.11	21.34±0.70	5.75±0.27	25.50±1.21

Mean ± SD values is expressed. Between each group and control group, there are not significant variations. Significant variance from control groups at $p < 0.05$. CO_2, carbon dioxide.

Table 4: Effects of *P. incurva*hydroalcoholic extract on liver biochemical parameters in male rats

Group \ Parameter		Total protein	Albumin	Globulin	TB	CB	ALP	ALT	AST	GGT
		g/L			µmol/L		IU/L			
control		58.10 ±1.13	8.7 ±0.45	49.31 ±1.31	0.66 ±0.11	0.68 ±0.12	184.00 ±16.31	44.15 ±1.21	60.30 ±10.30	3.2 ±1.42
P. incurva extract (mg/kg)	400	57.62 ±1.23	8.38 ±0.36	49.51 ±1.51	0.68 ±0.12	0.70 ±0.14	180.00 ±21.34	43.15 ±1.26	61.33 ±12.30	3.45 ±1.82
	800	60.00 ±1.14	8.51 ±0.62	50.61 ±1.12	0.72 ±0.13	0.71 ±0.12	185.13 ±17.41	44.73 ±1.34	62.53 ±8.66	3.25 ±1.23
	1600	58.00 ±1.47	9.31 ±0.65	49.36 ±1.12	0.69 ±0.12	0.69 ±0.15	183.17 ±19.45	45.73 ±1.14	62.73 ±11.52	3.00 ±1.19
	3200	59.05 ±1.36	8.44 ±0.37	50.68 ±1.13	0.71 ±0.00	0.70 ±0.18	186.16 ±15.73	44.63 ±1.61	60.76 ±9.83	3.03 ±1.28

Values expressed as mean ± SD. Between each group and control group, there are no significant differences. Significant difference from the control group at $p < 0.05$. ALP stands for alkaline phosphatase; AST stands for aspartate aminotransferase; CB is for conjugated bilirubin; GGT stands for G-glutamyltransferase; and TB stands for total bilirubin.

3.3. Biochemical results of oxidative stress markers (Serum MDA and GSH)

Rats treated with aspirin and ethanol had significantly higher levels of MDA in their stomach tissue than the control group. In contrast, rats treated with aspirin and ethanol had significantly lower levels of GSH in their stomach tissue than the control group. On the other hand, MDA level decreased and GSH level increased in AS and EtOH groups pretreated with *P. incurva* extract or ranitidine due to the significant protective effects of the *P. incurva* extract or the reference drug, Ranitidine. This protection might be ascribed to the the abilities of the plant extract to make stomach covering. The recorded values were represented in (**Table 5**).

Table 5: Effects of *P. incurva*hydroalcoholic extract and Ranitidine on MDA and GSH gastric concentrations in rats with aspirin and EtOH-induced ulcer

Group \ Parameter	MDA mmol/g tissue	GSH mmol/g tissue
Control group	803.67±1.0	11.82±1.34
AS group	1079.33±0.33	8.9±1.12
AS+PI group	848.3±0.00	11.9±0.00
AS+Ranitidine group	789.4±0.00	10.9±0.45
EtOH group	1860.8±21.2	8.89±0.24
EtOH+PI extract group	860.4±11.30	11.9±2.1
EtOH+Ranitidine group	830.7±1.69	10.21±1.15

Values expressed as mean ± SD. value at $p< 0.05$ compared to control group. There are no significant changes between groups AS+PI, AS+Ranitidine, EtOH+PI and EtOH+Ranitidinegroup compared with control group. There is a significant change between AS, EtOH groups and control group. AS, Aspirin; EtOH, Ethanol; PI, *Parapholis incurve*.

3.4. Macroscopic Evaluation of Gastric Lesions

Macroscopic appearance of the stomach of experimental rats confirmed that the control group exhibited intact stomach without lesions (**Fig. 1**). Aspirin treated group inside the glandular stomach, the most obvious results was linear hemorrhagic lesions and patchy light lesions observed hemorrhagic lesions. Results showed that rats

pretreated with ranitidine drug or *P. incurva* extract earlier than being given AS had drastically reduced areas of gastric ulcer formation as compared with AS treated group, the glandular area showed few dot-like and pale lesions without a linear hemorrhagic lesion. Ethanol produced extensive visible black hemorrhagic lesions of gastric mucosa. On the opposite hand, no haemorrhagic bands of ulcers or injuries were observed inside the gastric mucosa whilst the rats had been pretreated with Ranitidine or *P. incurve*(**Fig. 1**). The recorded results of ulcer index for all treated group have been represented in **Table 6**.

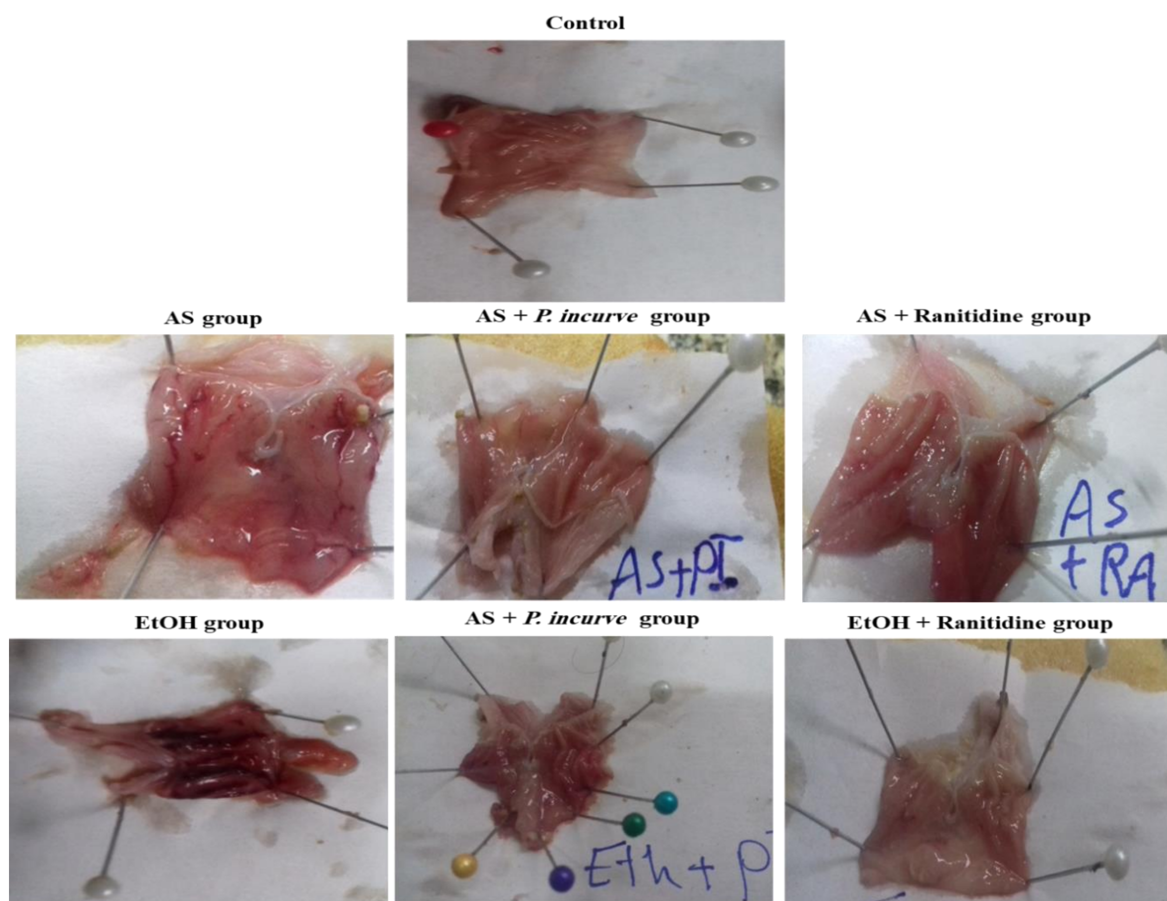

Figure 1: Every experimental group's stomach mucosa was examined in detail. Control group have normal gastric mucosa with prominent gastric rugae. AS group shows linear hemorrhagic lesions and patchy pale lesions accompanied hemorrhagic lesions. AS+*P. incurve* group shows few dot-like and pale lesions with no linear hemorrhagic lesions. AS+Rantidine group reveals gastric mucosa more or less as the control. EtOH group showed extensive visible black hemorrhagiclesions of gastric mucosa. No hemorrhagic bands of ulcers or lesions were seen in the stomach mucosa, as demonstrated by the EtOH+*P. incurve* group. EtOH+Rantidinereveals gastric mucosa with few faint red pinpoint spots within the gastric mucosa.

Table 6: Effects of *P. Incurva*hydroalcoholic extract on ulcer index in rats with aspirin and ethanol -induced ulcer

Groups	Mean ulcer index ± SE	Preventive index (%)
Control group	0 ± 0	0%.
AS group	23±1.45	0%.
AS+PI extract group	3.43±1.45	85.5 ± 2.5%.
AS+Ranitidine group	2.63±3.65	88.5 ± 1.5%.
EtOH group	79.87±3.55	0%.
EtOH+PI extract group	10.76±2.65	86.1 ± 1.6%.
EtOH+Ranitidine group	8.40±1.2	89.8 ± 1.5%

Values expressed as mean ± SD. value at $p < 0.05$ compared to control group. There are no significant changes between groups AS+PI, AS+Ranitidine, EtOH+PI and EtOH+Ranitidine group compared with control group. There is a significant change between AS, EtOH groups and control group. AS, Aspirin; EtOH, Ethanol; PI, *Parapholis incurve*

3.5. Histopathological results

Histological examination of H&E-stained sections of stomach of the control groupshows normal structure of mucosa with an intact epithelial surface, gastric pits, gastric glands, muscular layer and submucosa (**Figure 2-A**). Histopathology of ulcerated stomach of male albino rats receiving aspirin 400 mg/kg mg b.wt revealed many histological alterations such as loss of surface epithelial cells, numerous necrosis of gastric mucosa, submucosal vacuolation of the glandular portion, degenerative changes in the gastric gland and congesting blood vessels (**Figure 2-B**). PIprotected the histoarchitecture of stomach from the destructive effect of aspirin on the gastric mucosa as showing protection of the histological structure. However, only slight focal necrosis of gastric mucosa and submucosa, mild degenerative changes of gastric gland, and congesting blood vessels were observed (**Figure 2-C**). Also, rats treated with ranitidine in a recommended dose two hours before the Aspirin treatment showed a protection of the histological structure of mucosa though stomach section of animals which still suffered from mild focal necrosis of gastric mucosa and submucosa and closely packed gastric gland (**Figure 2-D**). The pronounced mucosal abnormalities observed in Stomach sections prepared from rats treated with EtOH 1ml/100g b.wt for gastric-ulcer induction revealed a loss of surface epithelial cells, necrosis with haemorrhage in gastric mucosa, and submucosa, degenerative changes gastric gland and congesting blood vessels in the submucosa (**Figure 2-E**). Stomach sections of rats that received orally (i.g.) the extract of PI two hours before ethanol treatment showed marked protection in the glandular architecture of the gastric mucosa comparable with those of the ethanol treated group since mucosal surface was more or less similar to the control group with mild erosion in some epithelial cells, moderate necrosis of gastric mucosa and submucosa, with closely packed gastric glands (**Figure 2-F**). Stomach sections of rats that received orally anti-ulcer drug in a recommended dose,(Ranitidine at 50 mg/kg b.wt)two hours before ethanol treatment showed remarkable protection as they showed almost normal structure of mucosa and submucosa with closely packed gastric glands (**Figure 2-G**).

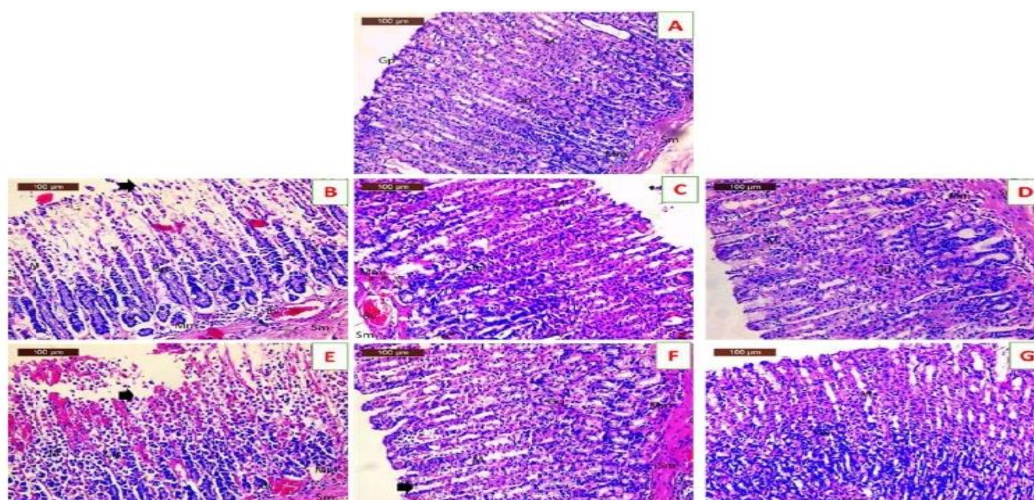

Figure 2: Photomicrograph of a section in the stomach from **(A) control rat** revealed normal shape of mucosa with an intact epithelial surface (M), gastric pits (Gp), gastric glands (Gg) muscular layer (Mm) submucosa (Sm), **(B) AS group** showing major histopathological changes consisting of loss of surface epithelial cells (arrowheads), numerous necrosis of gastric mucosa (M), submucosal (SM) vacuolation of the glandular component (V), degenerative alterations of gastric gland (Gg) and congestion of blood vessels (Bv), **(C) AS+PI extract group** showing histological structure improvement, the only mild focal necrosis of gastric mucosa (M) and submucosal (Sm), slight degenerations of gastric gland (Gg), and congesting blood vessels (Bv), **(D) AS+Ranitidine group** showing development of the histological structure with mild focal necrosis of gastric mucosa (M) and submucosa (Sm) and closely packed gastric gland (Gg), (E) EtOH group showing lack of epithelial cells (arrowhead), necrosis with haemorrhage in gastric mucosa (M), and submucosa (Sm), gastric gland degenerative (Gg), congesting blood vessels inside the submucosa (Bv), **(F) EtOH+PI extract** displaying mucosal surface had greater or less much like the control group with slight erosion in some epithelial cells (arrowhead), slight necrosis of gastric mucosa (M) and submucosa (Sm), with carefully packed gastric gland (Gg), (G) EtOH+Ranitidine showing almost regular shape of mucosa (M) and submucosa (Sm) with closely packed gastric gland (Gg). (H&E; scale bar=100 μm).

3.6. Periodic acid Schiff (PAS) stained sections results

The mucosal surface of the stomach in the control group was continuously covered with a thick PAS-positive magenta-red mucous film that extended to the pits, isthmus, and neck areas **(Figure 3-A)**. In contrast, the aspirin-treated group showed a total loss of PAS activity at the ulcerated areas and a localized loss of PAS reaction across the eroded surface epithelium **(Figure 3-B)**. The group that

received orally the *PI extract* two hours before AS revealed partly preserved surface epithelium and glandular epithelium with a marked protection in PAS-positive surface mucous cells. Even while the PAS-positive reaction was preserved in the neck and isthmus regions, it was not as noticeable as it was in the control group (**Figure 3-C**). PAS reaction within the gastric mucosa in rats that received ranitidine two hours before AS revealed good PAS-positive reaction appeared at the mucosal surface of the stomach, down to pits of the gastric gland (**Figure 3-D**). In Ethanol- treated group PAS stainrevealed complete loss of mucous secreting cells on the surface down to pits, isthmus and neck regions of the gastric gland (**Figure 3-E**). On the other hand, the pretreatment with the PI extract before ethanol administration revealed that PAS-positive surface mucous cells and neck mucous cells were markedly protected due to a mucin distribution (**Figure 3-F**). Also, stomach sections of rats that received ranitidine two hours before ethanol treatment revealed surface epithelium and glandular epithelium with moderate protection in PAS-positive surface mucous and neck mucous cells (**Figure 3-G**).

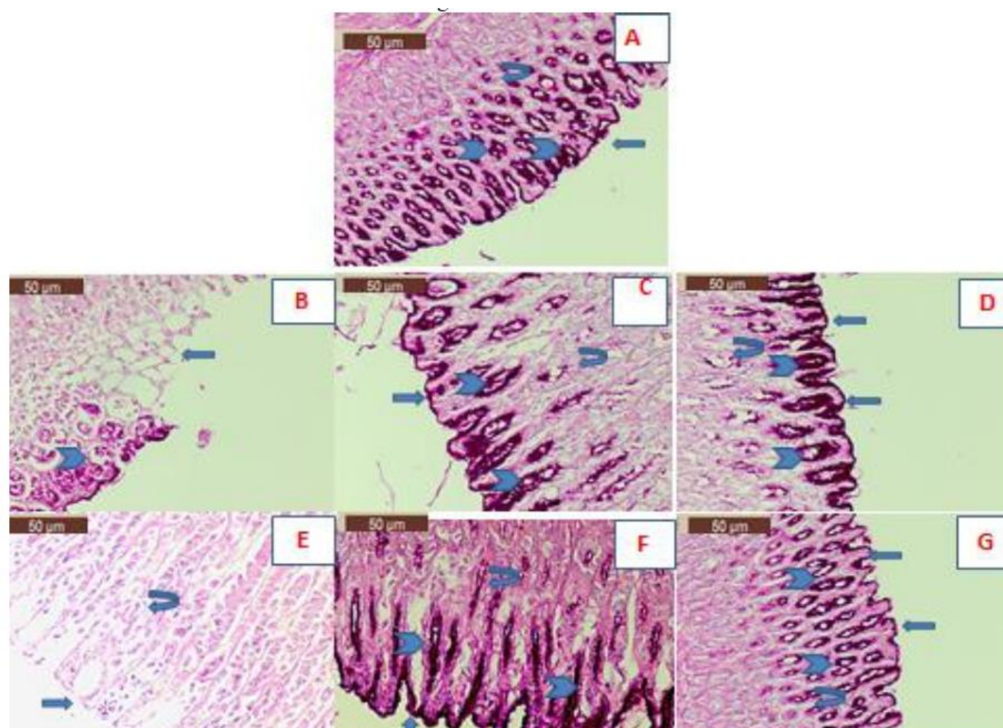

Figure 3: photomicrographs of each experimental groups stomach mucosa. **(A): control group** showed of strongly PAS-positive magenta red mucous film covering the mucosal surface (thick arrow), extending down to the pits, isthmus, and neck areas of the stomach glands (curved arrows). (**B): AS group** displayed a total loss of the PAS response at the ulcerated areas (thick arrow) and a localized loss of the PAS reaction across the eroded surface epithelium (arrow heads). **(C): AS+PI extract group** found that there was partial preservation at the isthmus and neck areas (curved arrows) and strong PAS +ve in the protected parts of the gastric glands over the surface epithelium (thich arrow), and gastric pits (arrow heads). **D): AS+Ranitidine group** revealed good PAS-positive reaction appeared at the mucosal surface of the stomach (thick arrow), gastric pits (arrow heads), but weak at isthmus and neck regions (curved arrows). (**E**): EtOH group revealed complete loss of mucous secreting cellsat the ulcerated region (thick arrows), gastric pits (arrow heads) and at isthmus and neck regions (curved arrows). **(F):EtOH+PI extract group** revealed PAS-positive surface mucous cells (thick arrows), moderately protected gastric pits (arrow heads), Weak PAS +ve reaction at isthmus and neck regions (curved arrows). **(G): EtOH+Ranitidine group** showed a PAS reaction closely similar to the control surface epithelium with strong PAS-positive magenta (thick arrow), down to pit (arrow heads), isthmus and neck regions (curved arrows) of the gastric glands.(Periodic acid Schiff reaction; scale bar=50 μm).

3.7. Mallory trichrome stain

Sections of the control group stained with Mallory trichrome were examined, the lamina propria between the stomach glands and the submucosa revealed thin and fine collagen fibers (**Figure 4-A**). In the aspirin-treated group, the gastric ulcer demonstrated an increase in the collagen fiber distribution in the submucosa and lamina propria between the gastric glands (**fig. 4-B**). Conversely, AS+PI extract showed similar levels of fine collagen fibers to the control group in the lamina propria between the stomach glands (**fig. 4-C**). When comparing the ethanol administration group to the mucosa tissue, there was a clear increase in the density and dispersion of collagen fibers in the mucosa layer in Mallory's trichrome stained sections (**fig. 4-E**). In group pretreated with PI extract showed a marked reduction in the

deposition of collagen fibers in mucosa layer as a slight amount of collagen fibers was seen throughout the mucosa layer (**fig. 4-F**). On contrary, the group pretreated the Ranitidine drug two hours before ethanol treatment revealed a few amount of collagenous fibers between mucosal glands and in the submucosa (**fig. 4-G**).

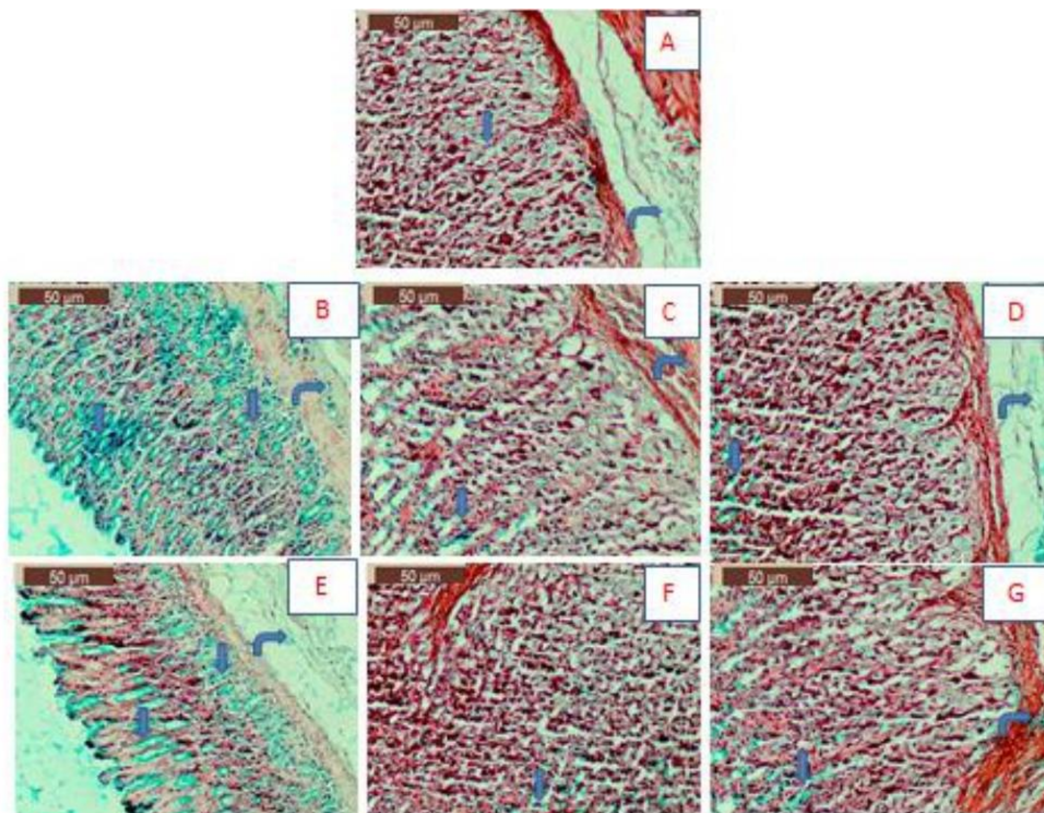

Figure 4: photomicrographs of each experimental group's stomach mucosa. **(A): Control group**, The submucosa (bent arrows) and lamina propria between the stomach glands (thick arrows) both displayed thin and fine collagen fibers. **(B): AS group's** gastric ulcer demonstrated an increase in the collagen fiber distribution in the submucosa (bent arrows) and lamina propria between the gastric glands (thick arrows). **(C): AS+PI extract** indicated similar amounts of fine collagen fibers to the control group in the lamina propria between the stomach glands (thick arrows). **(D): AS+Ranitidine**: exposes thin, tiny collagen fibers in the submucosa (bent arrows) and in the lamina propria between the stomach glands (thick arrows). **(E): EtOH treated group**: The density and dispersion of the collagen fibers are increased between mucosal glands (thick arrows) and in the submucosa (bent arrows). **(F): EtOH+PI extract** group show marked reduction in the deposition of collagen fibers in mucosa layer as a slight amount of collagen fibers was seen throughout the mucosa layer (thick arrows) and in the submucosa (bent arrows). **(G): EtOH+Ranitidine group**treatment revealed a few amount of collagenous fibers between mucosal glands (thick arrows) and in the submucosa (bent arrows). (Mallory's Trichrome; scale bar= 50 µm).

3.8. Immunohistochemical results

An immunohistochemical analysis of the gastric tissues of the control rats revealed caspase-3 (**Figure 5-A**) and cox2 (**Figure 7-A**) with negative stain. On the contrary, increased expression levels of Caspase-3 (**Figures 5-B &E**) and cox2 (**Figures 7-B &E**) were recorded in the aspirin treated group and strong brown cytoplasmic staining in EtOH group. Protection was recorded in Aspirin and ethanol groups pretreated with either PI extractor RA with a pronounced decrease in caspase-3 (**Figures. 5-C, D, F& G**) and cox2 expression (**Figures. 7-C, D, F& G**) in comparison with the Aspirin and ethanol groups.

Immunohistochemical analysis of the gastric tissues of the control rats revealed high expression of cox1 with strong brown cytoplasm (**Figures 6-A**). On the other hand, a weak brown cytoplasmic staining was recorded in Aspirin and ethanol treated groups compared with the control group (**Figures 6-B &E**). On the contrary, increased expression levels of cox1 with strong brown cytoplasmic staining were recorded in the ethanol and aspirin groups pretreated with PI extract and ranitidine drug in comparison with the Aspirin and EtOH groups (**Figures 6- C, D, F& G**).

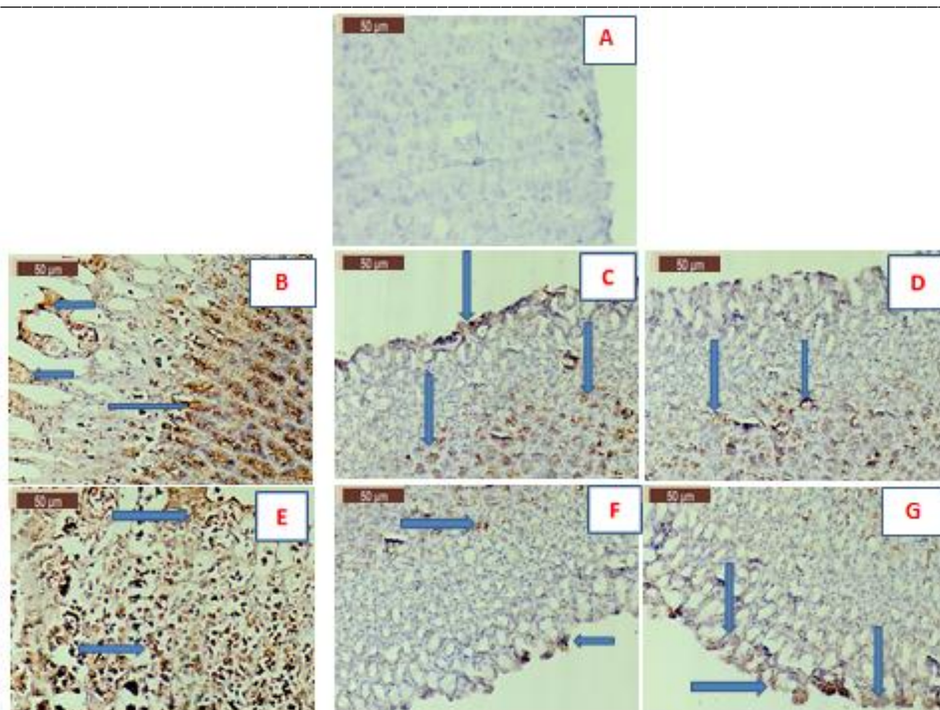

Figure 5: Photomicrographs of the gastric mucosa from all experimental groups show Caspase 3 immunohistochemical result. **(A): control group** revealed negative caspase- 3-positive stain (B): AS group reveal increased expression levels of Caspase-3 (thick arrows). (**C**): AS+PI extract group reveal pronounced decrease caspase-3 expression (thick arrows). **(D): AS+Ranitidine** revealed a marked decrease in caspase-3 expression (thick arrows). **(E): EtOH group revealed** Caspase-3 with strong brown cytoplasmic staining (thick arrows). **(F): EtOH+PI extract revealed** localized expression within the mucosa (thick arrows). (**G**): EtOH+Ranitidine revealed minimal caspase3 express (thick arrows) ion. (Caspase 3 immunohistochemical; scale bar= 50 µm).

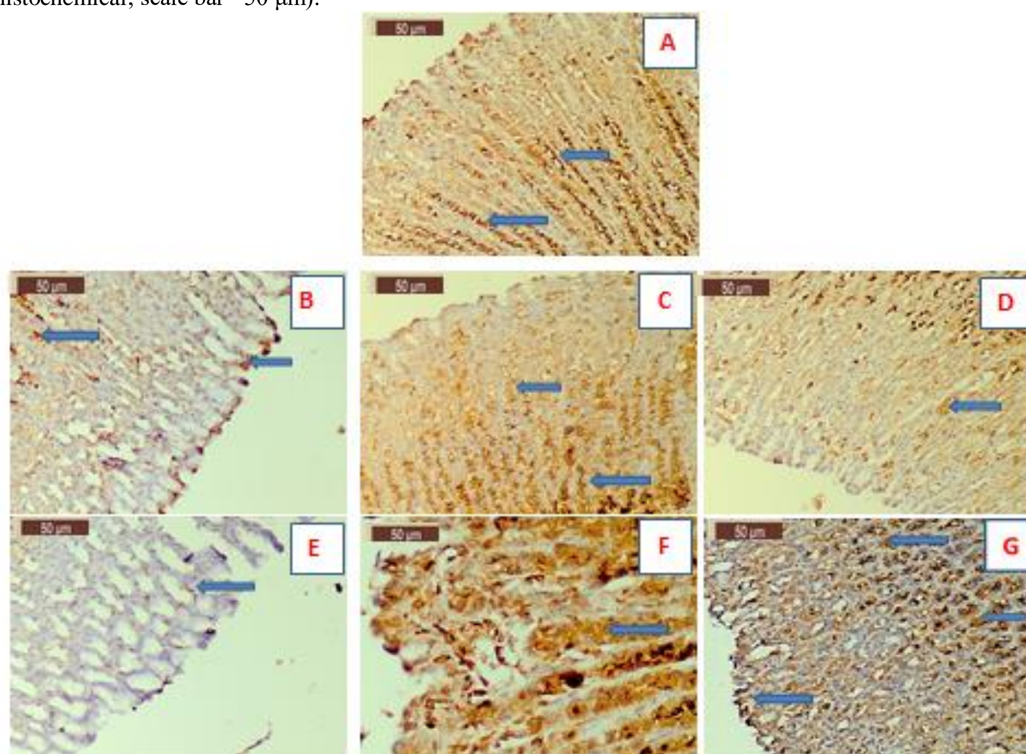

Figure 6: Photomicrographs of the gastric mucosa from all experimental groups show Cox1 immunohistochemical result. **(A): control group** revealed high amount of cox1 with brown cytoplasm(arrows). **(B): AS group showed** negative to weak brown cytoplasmic staining (arrows). **(C): AS+PI extract showed** increased expression levels of Cox1 (arrows). **(D)**: AS+Ranitidine revealed high expression levels (arrows). **(E): EtOH group showed** a scanty expression level of COX1 (arrows). **(F): EtOH+PI extract group** revealed a marked increase in amount of COX1 with strong brown cytoplasm (arrows). (**G**): EtOH+Ranitidine showed A strong expression levels (arrows). (COX1 immunohistochemical; scale bar= 50 µm).

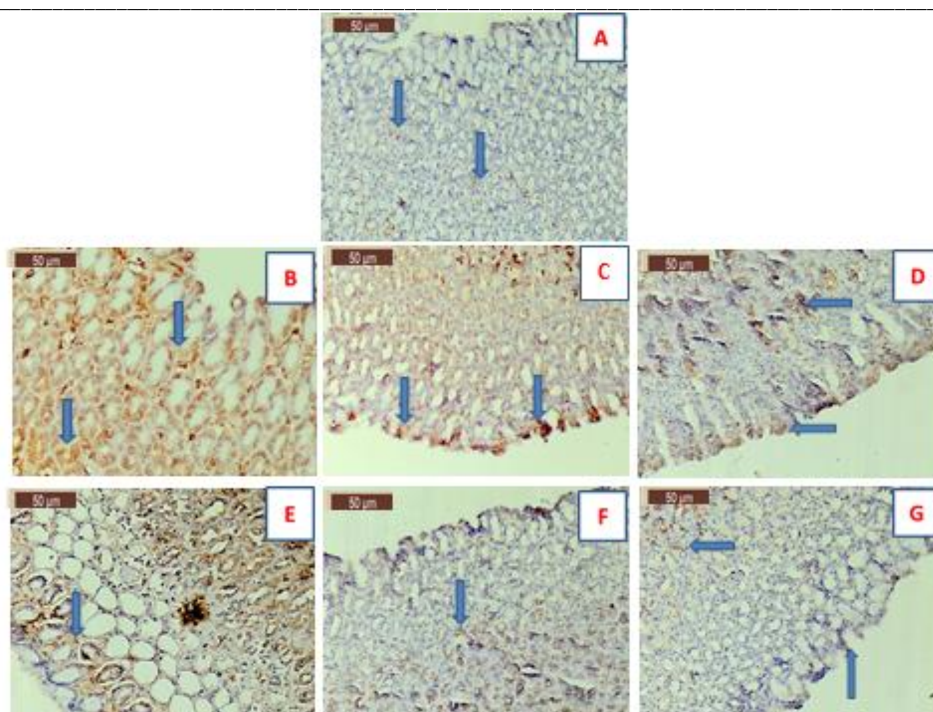

Figure 7: Photomicrographs of the gastric mucosa from all experimental groups show COX2 immunohistochemical result. **(A): control group** revealed negative to a slight expression of COX2(arrows). (**B**): AS group show increased expression levels of Cox2 (arrows). **(C): AS+PI extract** showed decreased expression levels of COX2 (arrows). **(D): AS+Ranitidine**revealed a weak expression level of COX2 (arrows). **(E): EtOH group** showed gastric ulcer with intense COX- 2 immunoreactivity with strong brown cytoplasm (arrows). **(F): EtOH+PI extract group** revealed a marked decrease in amount of cox2 (arrows). **(G): EtOH+Ranitidine** showed a weak expression level (arrows). (COX2 immunohistochemical; scale bar= 50 μm).

The results of the TPC and TFC estimations proves that this plant has a high concentration of flavonoids and phenolic compounds and two types of polyphenolic components. This finding was in fully agreement with the previous studies**[13]**. The reported data described the identification of the phenolic acids, gallic, caffeic, ellagic, coumaric, and cinnamic acids, and phenolic derivatives, caffeine, vanillin, propyl gallate, along with flavonoids, querectin, rutin, catechin, naringenin and 4`,7-dihydroxy isoflavone [**13**].The results of the present work recorded that oral feeding of *P. Incurve* extracts did not show any symptoms or signs of toxicity and no signs of toxicity or mortality in vivo and no variations in kidney and liver function to remark is consistent with the outcome of other research applied natural plant extracts **[29]**. Similarly, the herbal medication has antioxidant efficiency and causes neutralization of free radicals as pronounced with the aid of numerous co-researchers **[30]**.

Macroscopic appearance of the stomach of experimental rats showed that glandular stomach in Aspirin treated group most evident finding was linear hemorrhagic lesions and patchy pale lesions accompanied hemorrhagic lesionscompared with the control group that exhibited an intact stomach without lesions. **Asmaa [31]**detected macroscopic dark red hyperemic lesions in the gastric mucosa that were observed in gastric ulcer groups induced by aspirin. **Köroğlu et al.[32]** attributed to stasis in gastric blood flow, congestion of submucosal blood vessels and submucosal oedema which contribute to the development of the haemorrhages and necrotic aspects of tissue injury. **Jayan and Sanalkumar[33]**reported that aspirin induced ulcer at dose levels of 250-mg/kg, Ulcer index was 17.69 ± 0.12. Also, **Mohamed et al [34]** reported that the NSAIDs induced ulcer. Also, Macroscopic appearance of the stomach of experimental rats showed that ethanol treated group, produced extensive visible black hemorrhagic lesions of gastric mucosa. Observations received marked guide from the work of **Saeed et al.[35]**who said that ethanol caused gastric ulcer of animals resulted in gastric mucosal edema and large erosion, seen as elongated intense hemorrhagic lesions in comparison to healthy animals. On the opposite hand, administration of *P. Incurva* extract to rats before being given aspirin or ethanol had highly decreased areas of gastric ulcer formation in comparison with aspirin and ethanol treated organizations. This result may be attributed to antioxidant, anti-inflammatory and antiapoptotic sports of *P. Incurve* extracts which includes phenolic compounds like gallic acid and flavonoids like quercetin. This is in agreement with **Kahraman et al. [36]** who suggested that plants containing substances like quercetin and gallic acid have been effective in preventing ulcers, mainly

due to their antioxidant residences. **Kahraman et al. [36]** revealed that quercetin inhibited the development of mucosal gastric ulcers brought about by means of the use of ethanol. Also, our consequences confirmed that rats pretreated with ranitidine drug before being given aspirin or ethanol had notably reduced regions of gastric ulcer formation compared with aspirin and ethanol treated groups, the glandular region showed few dot-like and pale lesions. This is in agreement with **Naif et al. [37]**who reported that drugs that decrease gastric acid secretion and increase mucus secretion are effective in preventing the ulcers triggered by way of this method like ranitidine, performing as an antiulcer agent by means of an antisecretory mechanism via inhibition of gastric secretion. Also, it was documented that ranitidine pretreatment reduced ethanol aggression on gastric mucosa, nearby ulcers, erosions, and redness had been registered [**38**]. In the present work, histopathology of ulcerated stomach of male albino rats receiving aspirin 400 mg/kg mg b.wt. revealed many histological alterations such as loss of surface epithelial cells, numerous necrosis of gastric mucosa, submucosal vacuolation of the glandular portion, degenerative changes in the gastric gland and congesting blood vessels. This is consistent with earlier research showing aspirin caused severe ulceration, desquamation, and exfoliation of the epithelial cells on the surface **[39]**. Exfoliation of surface epithelial cells and discontinuity in mucosa ulceration caused by aspirin have been reported in similar findings. **[40]**. Additionally, the earlier documents described how lysosomal enzyme discharge that builds up in parietal cells may also cause these vacuoles. Additionally, they explained how disturbed membrane permeability may cause cellular vacuolation due to oxygen-free radicals that enhance the delivery of electrolytes and water into the cells, causing cells to swell and organelles to be destroyed, resulting in cytoplasmic vacuolation **[41]**. Furthermore, similar results suggested that the inflammatory cellular infiltration seen inside the ulcerated group may be caused by interactions between neutrophils and vascular endothelial cells, with increased adhesion between the two groups in both the mesenteric and gastric venules. This interaction ends with the discharge of numerous inflammatory mediators from the activated neutrophils, which is likely involved in the pathophysiology of gastric mucosal injury **[42]**. These alterations were linked to the direct mucosal infection caused by aspirin. This occurs when the stomach's acidic environment instantly transforms aspirin and the majority of NSAIDs into more lipid-soluble unionised acids and forces their buildup in the gastric mucosal cells. This changes the permeability of the cell, allowing acid and pepsin to diffuse back into the mucosa and causing ulceration **[43]**. Low oxygen anxiety and the depletion of adenosine triphosphate (ATP) generation within the cells have been identified by Rubin and Strayer, Bjarnason et al., and Radwan et al. as the primary link connecting the detrimental effects of NSAIDs. As a result, the ATP-structured Na+/K+ pump interferes with cellular membrane permeability, causing sodium to accumulate in the cell and an osmotic advantage of water alongside intracellular calcium inflow from the extracellular fluid and its release from intracellular stores, which would activate proteases, phospholipases, and endonucleases, causing surface epithelium exfoliation **[44-46]**. Stomach sections of rats given orally the hydro-ethanolic extract of *P. Incurva* two hours earlier than the aspirin showed protection in comparison with aspirin handled group, the mucosa become nearly similar to that of control group. This result may be attributed to antioxidant, anti-inflammatory and antiapoptotic function of *P. Incurva* extract this is excessive in antioxidants, group of phenolic compounds including Rutin. This is in agreement with **Olaleye et al.[47]**who reported that the flavonoid substances such as Rutin showed antiulcerogenic and recuperation action within the persistent ulcer model, together with the ones triggered with the aid of acetic acids. Flavonoids protect the gastric mucosa in numerous experimental models of ulcers and their movement is related to many mechanisms, which include cytoprotective, antisecretory, immunoregulatory, antioxidants **[48].** The flavonoids, such as rutin, quercetin and its glycosides further to catechin and gallic acid, played significant anti-inflammatory and antioxidant roles, Rutin was described to have the capability for protection the gastric mucosa in all experimentally prompted gastric lesions **[49]**. Stomach sections of rats that received orally Ranitidine two hours before ethanol treatment showed marked protection in the glandular architecture of the gastric mucosa comparable with that of the control group. According to studies on ranitidine's histopathological protection against ethanol-induced gastric ulcers, the ulcer healed with few inflammatory cells and in a dose-dependent manner by activating pepsinogen, protecting the gastric mucosa, and blocking the parietal cells' H_2 receptors **[51,52].** In aspirin treated group, gastric ulcer revealed a focal loss of PAS reaction over the eroded surface epithelium, and complete loss of the PAS reaction at the ulcerated regions. Similar observations were described that the stomach ulcers is attributed to the decrease in the area percent of PAS-stain as a histochemical response with depletion of mucus of surface of the distorted gastric glands in the ulcerated rats **[42]**. That could be attributed to the direct cytotoxic effect of aspirin on gastric mucous secreting cells with reducing of their numbers, diminishing mucus manufacturing and mucus barrier disorder that's connected to the oxidative stress and the inflammatory infiltration. As compared to the Aspirin or ethanol treated groups,

the ranitidine drug pretreatment group exhibited a drop in blood MDA levels and an increase in serum GSH. Rats with stomach ulcers were shown to have elevated MDA levels; however, the groups receiving ranitidine showed a significant reduction in these levels. Ranitidine's ability to lower MDA and raise GSH levels demonstrates its anti-oxidant properties against oxidative stress generated by aspirin **[78-79].** In ethanol treated group, gastric ulcer significantly revealed complete loss of mucous secreting cells on the surface down to pits, isthmus and neck regions of the gastric gland compared with stomach of animals in control group. This is in agreement with **Betül et al. [53]**who reported that histochemical examination in stomach sections prepared from rats treated with absolute ethanol 1ml/100g b.wt for gastric-ulcer induction for the mucopolysaccharides by using PAS stain significantly revealed complete loss of mucous secreting cells on the surface down to pits, isthmus and neck regions of the gastric gland. On the other hand, in the present study the ethanol pretreated with extract of PI revealed PAS-positive surface mucous cells. In the PAS staining evaluation, this had a look at validated that *P incurve* heightened the content of glycoprotein inside the gastric mucosa. These impacts will be due the phenolic content functions, quercetin as antioxidant content in PI extract. These results were consistent with the study conducted by **Alvarez et al. [54]** in which the *Bidenspilosa* extract exhibit significant antiulcer activity via the reduction of the amount of gastric juice, acid secretion, and pepsin secretion in rats with pylorus ligation and attributed to the presence of flavonoids.

The histochemical evaluation of Mallory's trichrome-stain in aspirin and ethanol treated groups, the gastric ulcer showed an increase in the collagen fiber density and distribution in mucosa layer compared to the control group. These findings align with those reported by **Fatma et al. [40]**, who reported that aspirin-treated rats had increased collagenous fibres in both the submucosa and the gland core, with some collagenous fibres extending between the basal portions of the glands. Following the administration of aspirin and ethanol, there was a significant rise in the amount and distribution of fibres of collagen in Mallory's trichrome stained sections. **Mahmoud & Abd El-Ghaffar [55]** explained that fibrosis is essentially the result of overproduction of reactive oxygen species, dysregulation of apoptosis, and increased expression of proinflammatory cytokines such as TNF-α, C-reactive protein, transforming growth factors (TGF-α or -β), and nuclear transcription factor–Kappa-B (NF-κB). This activation of myofibroblasts allowed for the deposition of collagen and extracellular matrix components.

On the other hand, no collagenous fibers were detected in the submucosa and bases of the fundic glands in both stomach sections of groups treated with orally *PI* extract and the ranitidine drug and a marked reduction in the deposition of collagen fibers in mucosa layer as a slight amount of collagen fibers was seen throughout the mucosa layer of rats treated with *PI* extract revealed no collagenous fibers between mucosal glands. These impacts were due to PI extract that include highly antioxidant groups of phenolic compounds. Catechin as example as main component of PI extract could improve histological ulceration induced by either aspirin or ethanol. Catechin and its gallate derivatives are monomeric flavanols with amazing antioxidant and anti-inflammatory sports present in green tea **[56]**. Mallory trichrome stained sections of the control group, gastric ulcer induced group discovered few collagenous fibers in the submucosa, lamina propria and bases of the fundic glands. While increase in the quantity of collagen fibers become visible within the aspirin pretreated with GSE institution that may be explained by means of begin of manner of ulcer healing. **Naif et al. (2022)[50]** defined the safety mechanism of ranitidine, he reported that agents' lower gastric acid secretion and increase mucus secretion are powerful in stopping the ulcers precipitated by way of this approach like ranitidine, performing as an antiulcer agent with the aid of an antisecretory mechanism through inhibition of gastric secretion.

In the present study the immunoreactivity in the stomach sections revealed Caspase-3 expression was up-regulated in aspirin and ethanol groups in comparison with control group. On the other hand, immunohistochemical results revealed a low expression of Caspase-3 in aspirin and ethanol pretreated group by PI extract in comparison with aspirin and ethanol groups. This protective effect is due the significant antioxidant capabilities of flavonoids contents. The results of previous study by **Dan Zhou et al. (2020)[57]**that revealed the gastroprotective impact and the principal mechanism of gallic acids which is a phenolic compound located in many medicinal plants on ethanol-caused gastric ulcer in rats. Caspase-3, a key mediator for apoptosis, a key mediator for apoptosis loss of life in stress-caused ulcer rat model, expression was reported to be strongly regulated via the phenolic acids **[57-59]**. The documented HPLC analysis of PI extract described that the highly antioxidant and anti-inflammatory mediators, including flavonoids and phenolic acids, represented with high concentrations **[13-60]**.

The evaluated immunoreactivity of COX-1, and COX-2 in the stomach sections of aspirin and ethanol groups revealed a reduction of COX-1, and increased COX-2 expression. On the other hand, a low expression of COX-2 and a high expression of COX-1, levels were detected in aspirin and ethanol pretreated groups by PI extract in comparison with aspirin and ethanol groups.**Sengul and Gelen[61]** also found out that NSAIDs induced an increase in gastric COX-2 in contrast to the controls. A similar view was announced by **Ahmed et al. [34]** who recorded that the rats received aspirin as aspirin

considerably increased the immuno-expression of COX-2. This was supported also by **Yomna and Eman[70][62]** who found that Spirulina cause COX-1 immunoreactivity inside the gastric mucosa due to the antioxidant components, such as polyphenols and phenolic acid contents. According to **Vivatvakin et al. [63]**who declared that flavonoids protect the gastric mucosa from damage caused by NSAIDs, suggesting that flavonoids can act through a cytoprotective mechanism (as an instance with the aid of stimulating prostaglandins and mucus and bicarbonate secretion), growing the protecting factors and preserving the integrity of the gastric mucosa. MDA level in stomach tissue was markedly enhanced in ethanol and Aspirin treated groups. On the other hand, a significant decrease in serum levels of GSH compared with control group**. Sidahmed et al. [64]**supported the above results as he found out that Aspirin has oxidative stress, which results in elevation of MDA with reduced GSH level that are important antagonists of the intracellular antioxidant protection system. **Yakout et al.[65]**indicated that gastric MDA was accelerated and gastric GSH extremely reduced in ethanol-caused gastric ulcerative rats. **Antonisamy et al. [66]** found out that ethanol causes oxidative stress via increase in lipid peroxidation that has a major function within the pathogenesis of acute gastric ulcers. The production of reactive oxidative species (ROS) in gastric mucosal tissue continues at a regular degree, due to the equilibrium among pro-oxidant and antioxidant structures. Conversely, the equilibrium is altered in lots of instances, along with consuming alcohol **[66]**.

In the current investigation, pretreatment of Aspirin or ethanol with PI extract showed an extensive decrease in MDA levels and an increase in levels of GSH compared with control group. Such protective function of PI extract could be explained through its antioxidant effect. Catechin and naringenin derivatives reported as main flavonoids in PI extract are responsible for such antioxidant activity. Similar findings have been discovered by **Zaki et al. [67]**who reported that flavonoids of green tea, in particular catechin have antioxidant function. These effects are confirmed by **Horiuchi et al. [68]** who stated that distinct experimental gastric mucosal lesions stimulated in animals using HCl/EtOH, NSAIDs with *B. Pilosa* extract protected the gastric tissue against peroxidative stress and decreased the index of lipid peroxidation in gastric mucosa. The key components in *B. Pilosa* are seven flavonoids, catechin and six caffeic acid derivatives which have antioxidant activities.

4. **Conclusion**

Therefore, it was possible to draw the conclusion that the protection with PI extract therapy shown therapeutic promise in preserving mucosal stomach changes in these rat models of aspirin and ethanol-induced ulcer. According to this study, PI extract could prevent stomach mucosal damage caused by aspirin and ethanol. A treatment for gastric ulcers might be possible because of the protective action of *P. incurve* extract, which is mediated via enhancing cytoprotective and antioxidant defences as well as reducing oxidative stress and inflammation. These antiulcer potencies of this plant were directly attributed the singular and/or synergetic effects of its phenolic and flavonoid components.

Conflicts of interest

There are no conflicts to declare.

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