Evaluation of gastroprotective effect and wound healing potential of *Cibotium barometz* in animal models

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- Research Methodology
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Introduction

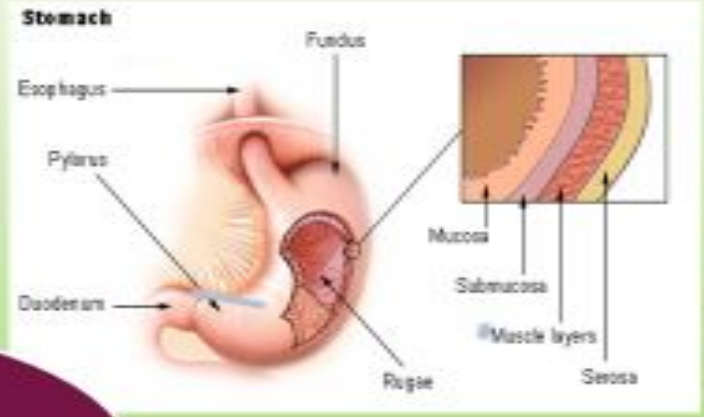
Gastric Ulcer:

- One of the most essential ailments of Gastrointestinal Tract in the world ,which is Gastric ulcer, **becomes a medical-social problem globally** due to increasing of its morbidity and mortality.
- It is the new "**plague of the 21st century**" that has been referred by some authors.

Continue Gastric Ulcer:

The stomach

- Primary organ of the digestive tract.
- Releases proteases and HCl.
- **4 layers of the stomach.**



Gastric ulcer

- **Imbalance** between aggressive factors (endo & exo) and protective physiological mechanisms.

Causative factors

- NSAIDs, alcohol, *H. pylori*, **infection**, cigarette smoking
- **Stress**, hormones ,drugs, food.
- **HCl & pepsin.**

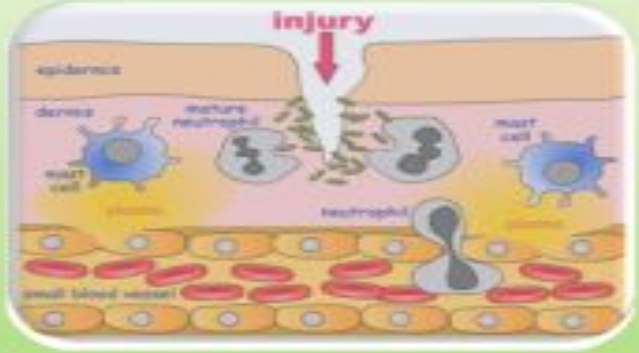
Continue of *Gastric Ulcer:*

- Nowadays, many **researchers** are carrying out on this common pathologies to lessen its complications.
- Sometimes **anti-acid drugs** are **unsuccessful** and impair the absorption of acid gastric medium requirements of calcium, iron, magnesium and vitamin B12 for bioavailability.
- **Medicinal plants** can assist in **ulcer healing, in preventing recurrence** and were used as alternative treatments.

Wound Healing

Four phases:

1) Haemostasis
2) **Inflammation,**
3) Proliferation
4) Tissue remodelling/ resolution.



Dynamic process and **auto process** involving cellular, molecular, biochemical and physiological phenomena for injured skin.

* These phases and their bio physiological functions must occur in **the proper sequence**, at a **specific time, and continue for a specific duration at an optimal intensity depending on the extent of wounding for wound healing**

*Has an **epidermal and a dermal layer.**
1) The **epidermis** (keratinocytes)
2) **dermal** part fibroblasts and extracellular matrix (ECM)

Wound Healing Treatment:

- There are **different drugs** and **vehicles** have been used locally or systemically to accelerate the healing. Including antibiotics, gels, bandages and mechanical devices that reduce evaporation of water.

- Many herbs and **medicinal plants** have been used for **wound healing** in folk medicine with **less undesired consequences** and **repair shortly**

Medicinal Plants:

- The use of **herbal medicines** has been **increasing last decades** and more widespread of traditional medicines.

- Many people in the world use the herbs as a **preventative** medicine.
- Some of medicinal herbs are utilized for **treatment** some ailments

Continue to Medicinal Plants:

- The medicinal plants have various parts and **different medicinal properties**. Therefore, a lot of food supplements and many drugs of traditional herbal medicines have being produced.

- In many Asian countries, **leaves** were applied to treat **cuts**, rheumatisms & **ulcers**.

- Medicinal herbs and plants have **natural antioxidant capacity** which leads their consumption to best health and nutrition.

Antioxidant activity of medicinal plants:

- Antioxidant is a **chemical substance** that can prevent the initial or propagate process of oxidative chain reaction.
- It can directly restrict the lipid and other molecule's oxidation that cause **diseases**.
- It can donate an electron to a free radical and convert it to a harmless molecule.
- **Medicinal plants** and herbs possess **more potent antioxidant** activity than fruits and vegetable.

Cibotium barometz

Common Names:

Golden Hair

It is a **tropical and subtropical plant**

Kingdom:	Plantae
Division:	Pteridophyta
Class:	Pteridopsida
Order:	Cyatheales
Family:	Dicksoniaceae
Genus:	*Cibotium*
Species:	*C. barometz*

C. barometz Leaves

- Has **antibacterial** activities.
- It is **anti-inflammatory**, used for the treatment of rheumatism and sciatica.
- Has **antioxidative tyrosinase inhibiting** that inhibit enzymatic browning in plants, can also be used in medicinal and cosmetic whitening agents
- The hairs are applied on **cuts and wounds** to stop bleeding.

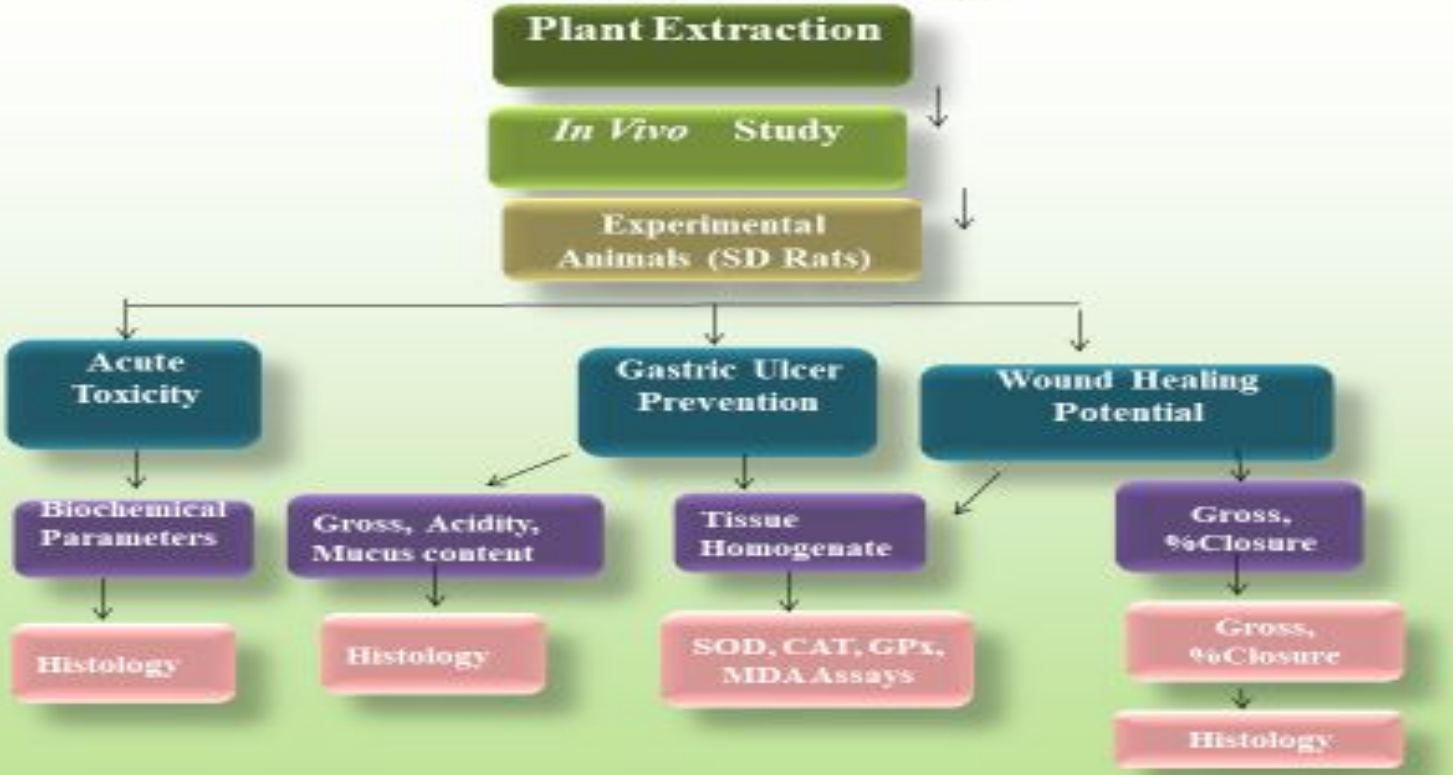
Objectives

1) To evaluate the **gastroprotective effect** of ***Cibotium barometz*** in experimental *rats*.

2) To evaluate the **wound healing potential** of ***Cibotium barometz*** in experimental rats.

Research Methodology

Experimental Design



Design of work

Plant Extraction

Collection, washing

Drying, Grinding

Maceration 4-5 days, Ethanol 95%

Filtration, Evaporation (Buchi Rotary Evaporator R-215)

Keep on -20°C in freezer

In Vivo Study:

(OECD guidelines)

Acute Toxicity Test

36 SD Rats

18 male Rats

18 female Rats

6 Rats (5mL/kg) 10% Tween 20	6 Rats (2 g/kg) 2 mL/kg Extract	6 Rats (5 g/kg) 5 mL/kg Extract	6 Rats (5mL/kg) 10% Tween 20	6 Rats (2 g/kg) 2 mL/kg Extract	6 Rats (5 g/kg) 5 mL/kg Extract

Sacrificing all Rats after 14 days

Liver & Kidney (Histology)

Blood Serum (Biochemical Tests)

In Vivo Study:

Gastro protective study

Group 1	Group 2	Group3	Group 4	Group5
6 Rats Vehicle 10% Tween 20	6 Rats Ulcer 10% Tween 20	6 Rats Omeprazole 20 mg/kg	6 Rats 250 mg/kg Extract	6 Rats 500 mg/kg Extract
After 1 hr.				
10% Tween 20 5 mL/kg	Ethanol 5 mL/kg	Ethanol 5 mL/kg	Ethanol 5 mL/kg	Ethanol 5 mL/kg

Sacrifice all rats after 1 hr., remove Stomach

- Gross
- Histology (H&E) staining
- PAS staining, Immunohistology (BAX, HSP70) staining
- Mucus content, Acidity
- Gastric tissue Homogenate
  - SOD, CAT, GPx, MDA

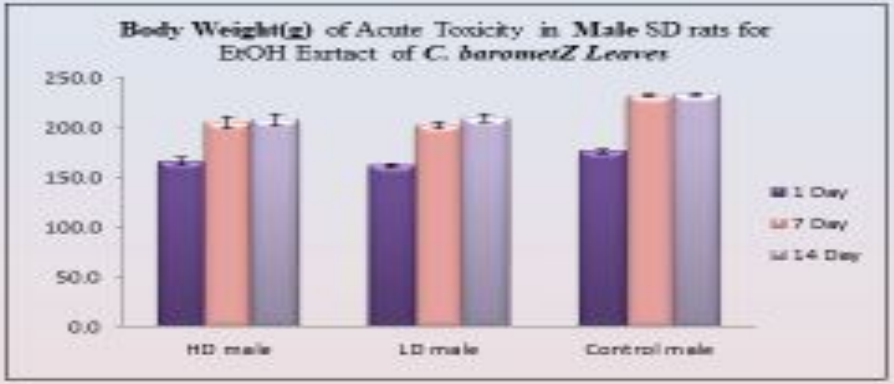
In Vivo Study

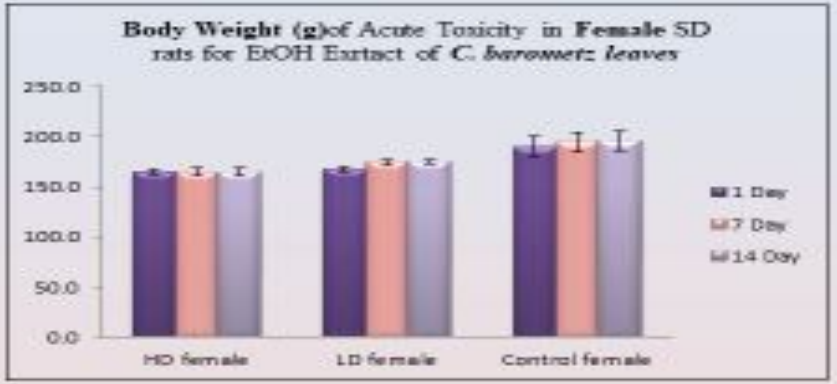
Statistical analysis:

- All data was analysed by using **SPSS programme** for windows version 20. The statistical significance of differences between groups was assessed using **one-way ANOVA (**Analysis of Variance**)** followed by post hoc Tukey's multiple comparison tests.

Results and Discussion

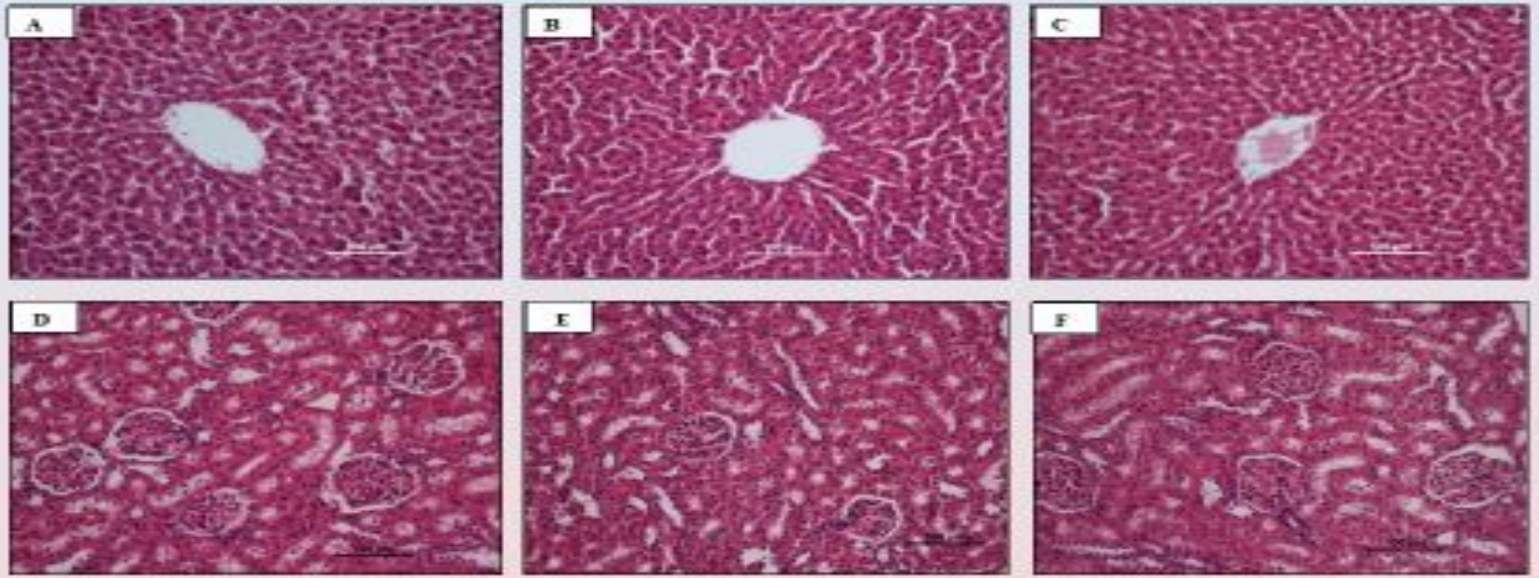
Figure 1. Acute Toxicity of *C. barometz* leaves ethanol extract on the **body weight(g)** of the **male** and **female SD** rats.





- Values were expressed as **mean ± S.E.M**. There are **no significant** differences between groups. Significant value at $p<0.05$.
- All SD rats that were treated with the *C. barometz* ethanol extracts showed **no mortality or toxic symptoms of this experiment**. There were **no body weight alterations** as showed in this Figure, **no abnormal physiological or behavioural changes** at **2 g/kg** and **5 g/kg** doses during 14 days that compared to vehicle group CMC 0.5%. There are **no significant** differences between groups.

Figure 3. Histological sections (Haematoxylin and Eosin stain) of the **liver and kidney** of the **acute toxicity test** of ***C. barometz*** leaves ethanol extract on the experimental SD rats.



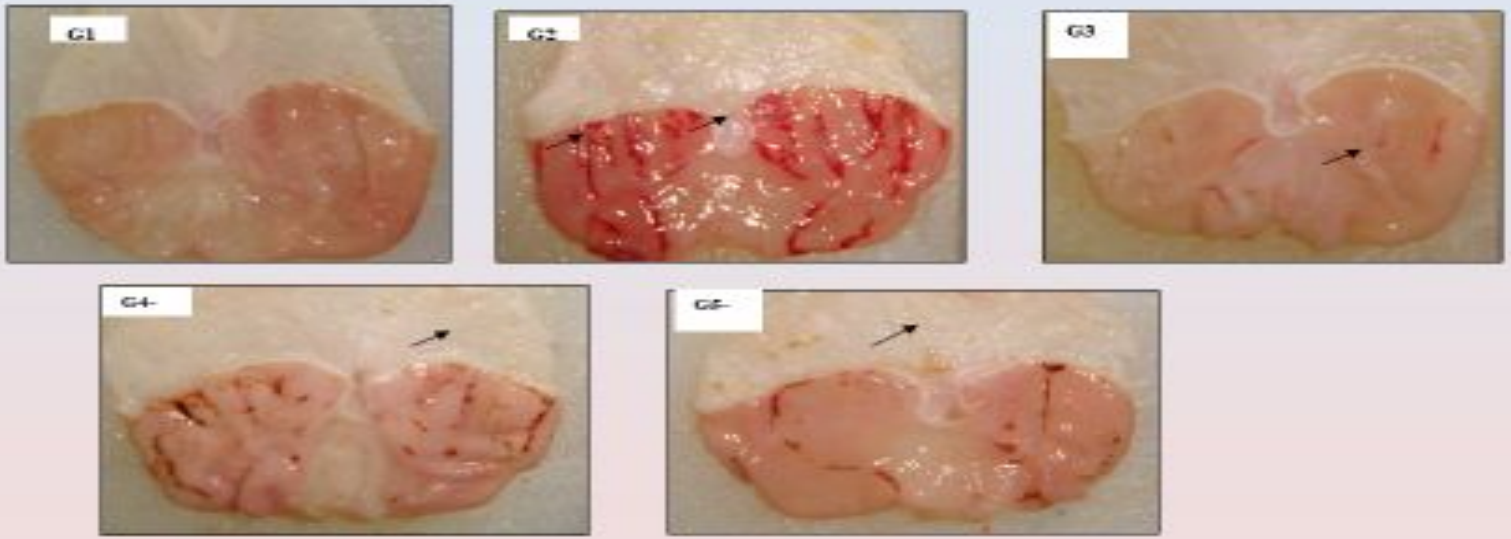
(A, D) Rats treated with 5mL/kg of the **vehicle (CMC 0.5%)**. **(B and E)** Rats treated with **2000 mg/kg** (5mL/kg) of the *C. barometz* extract. **(C and F)** Rats treated with **5000 mg/kg** (5mL/kg) of the *C. barometz* extract. **No significant changing and no lesions appear in the structures of the livers A, B, C and kidneys D, E, F between the treated and normal control groups** (20 × magnifications).

Table 3. The effect of *C. Barometz* extracts on the **Mucus weight, PH of gastric content, ulcer area, %inhibition** of ulcer area in the stomach samples collected from all experimental animals.

Animal Group (SD rats)	No Group	Pre-Treatment 5ml/kg	Mucus Weight (g)	PH (Acidity)	Ulcer Area (mm2)	Inhibition %
Normal	G1	10% Tween20	2.29 ± 0.15*	7.17 ± 0.38*	-	-
Ulcer	G2	10% Tween20	0.76 ± 0.15	2.77 ± 0.28	801.60 ± 35.65	-
Omeprazole	G3	20mg/kg	1.93 ± 0.06*	5.72 ± 0.49*	96.00 ± 20.42*	88.02%
C. barometz Leaves extract	G4	(250mg\kg)	1.41 ± 0.18*	5.86 ± 0.42*	150.00 ± 21.39*	81.29%
	G5	(500mg\kg)	1.78 ± 0.17*	5.20 ± 0.47*	126.0 ± 5.50*	84.28%

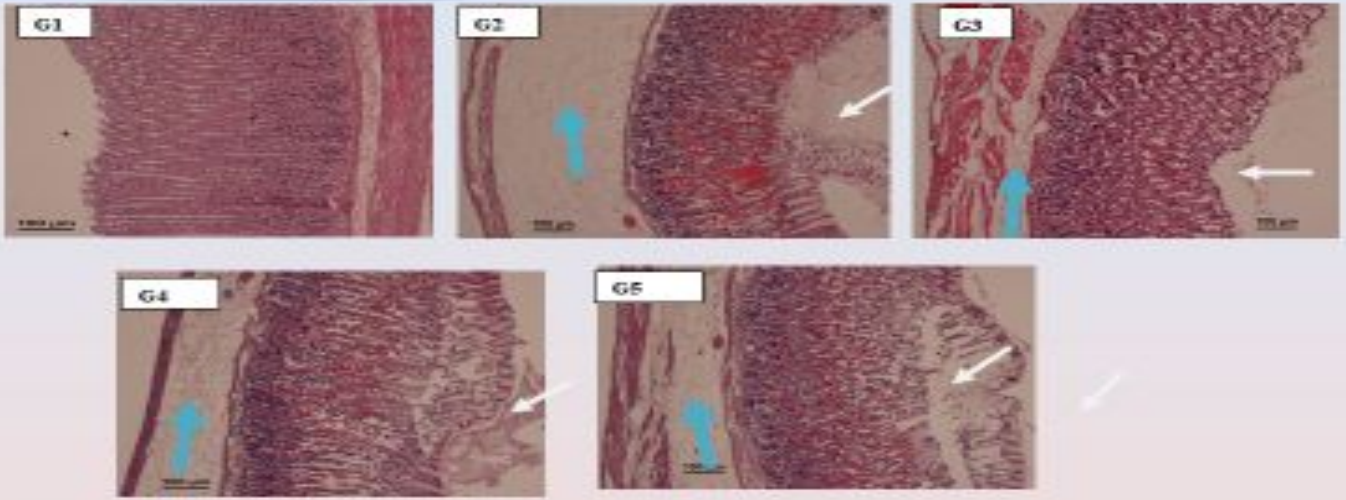
- All Data values are expressed as mean ± SEM.
- * Indicates significance at $p < 0.05$ compared to ulcer group.

ure 5. The effect of *C. barometz* on **the macroscopic appearance of the gastric mucosa** in ethanol-induced **gastric mucosal lesions** in **male SD rats.**



(G1) (Normal control group); **(G2) (Ulcer control group)**; **(G3) (Omeprazole)**; **G4** (250 mg/kg), **G5** (500 mg/kg). *C. barometz* (ethanol extracts.

Figure 6. Histological evaluation (H&E staining, 20×)of Ethanol-induced **gastric mucosal damage** for *C. barometz* on SD rats experiment.



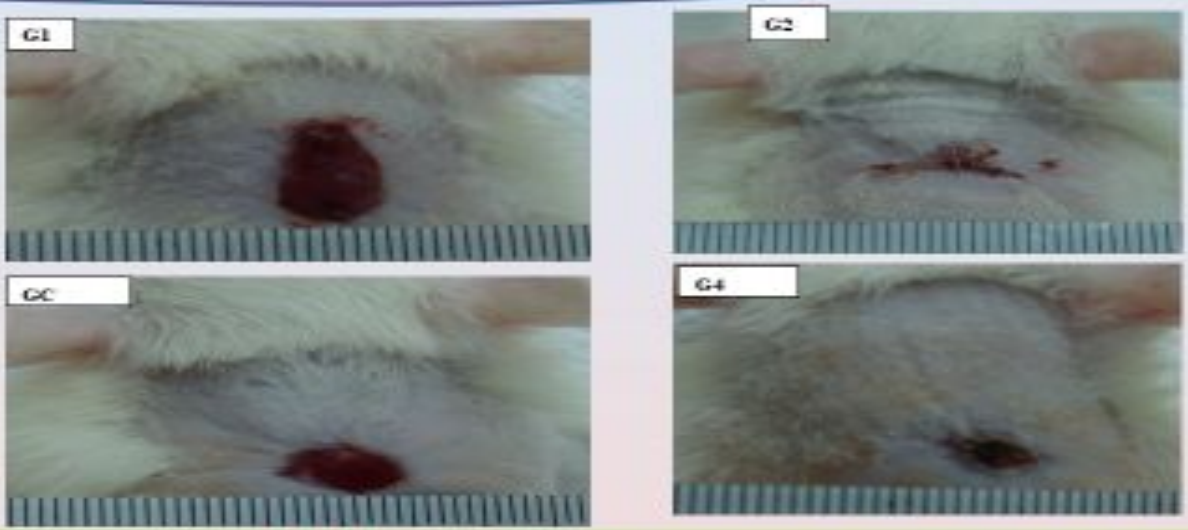
- **(white arrow)** for absence of ulcer area or lowering. **(blue arrow)** for submucosal edema and leucocyte infiltration.
- **G1.** 10% tween20 **(Vehicle control)** has **intact surface mucosal epithelium**. No lesion. **G2.** 10% tween20 **(ulcer control)** has **severe disruption** of the surface epithelium and necrotic lesions and extensive edema of the submucosal layer and leukocyte infiltration.**G3. Omeprazole (20 mg/kg)** has **mild disruption** of the surface epithelium mucosa, and there is a reduction in the submucosal edema and leucocyte infiltration.
- . **G4.** *C. barometz* **(250 mg/kg)** has **mild disruption** of the surface epithelium.
- **G5.** *C. barometz* **(500mg/kg)** has **mild disruption** of the surface epithelium.

Table 4. The effects of *C. barometz* leaves on the **percentage of wound healed and wound area** on **5th Day** and **10th Day** post wounding for experimental male normal SD rats.

Groups	Doses	Wound Area mm²		Closure %	
		5th Day	10th Day	5th Day	10th Day
Vehicle (CMC 2%)	(0.2ml/kg)	153.3±7.4	65.3±10.6	51.2	79.2
Intrasit gel	(0.2ml/kg)	138.0±11.3	27.7±2.3*	56.1	91.2
C. barometz Leaves extract	(0.2ml/kg) 100 mg/kg	228.0±11.7*	47.2±12.6	27.4	85.0
	(0.2ml/kg) 200 mg/kg	172.0±7.4	32.0 ±9.0*	45.2	89.8

- All values are expressed as Mean ± SEM. * means significant (P <0.05) pre-treatment compared to vehicle (CMC 2%)

Figure 8. Macroscopically appearance of excision wound healing area treatment 0.2 ml of *C. barometz* at **Day 10-post-wounding** on experimental male SD rats.



- **(G1) 0.2 ml of 2% CMC vehicle(Normal group). (G2) 0.2 ml of intrasite group (Reference group). (G3) 0.2 ml of 100 mg/ml of *C. barometz* treated group treated group. (G4-C) 0.2 ml of 200 mg/ml of *C. barometz* treated group.**. Grossly, wounds dressed with *C. barometz* or with reference control group (Intrasit gel) showed considerable signs of dermal healing that healed **faster compared to group received the vehicle control (2% CMC)**.

Conclusion

Conclusion:

- *C. barometz* leaves have **antioxidant activities *in vitro*** which means high capacity of antioxidant agents that can play as scavengers of free radicals.
- Acute toxicity test of *C. barometz extract* did **not show any signs of toxicity** and **no mortality *in vivo*** that means the plants are **safe**.
- *C. barometz* extract revealed antiulcer effects against ethanol-induced **gastric lesions in the animal model significantly and dose-dependently**. The gastroprotective effect could be associated with the effective direct radical scavenging activity and raising of the cellular antioxidant activities of gastric SOD, CAT and GPx levels along with depression of lipid peroxidation (MDA).
- *C. barometz* extract are **effective in acceleration of wound healing** in rats may be due to their antioxidant activities that protects biological molecule from oxidative damge and raising the deposition of collagen and angiogenisis in granulation tissue.

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Thank You