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Original Research Article

Pharmacological Evaluation and Phytochemical Screening of *Dactylorhiza Hatagirea* on Anti-Ulcer Activity

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Abstract

In the present work, the acute toxicity was carried out based on OECD-423 rules for the chloroform extract of *Dactylorhiza hatagirea*, proving that the doses of 250 and 400 mg/kg did not indicate any sign of toxicity or mortality. Hence, these doses of the concentrate were chosen for the assessment of anti-ulcer activity. *Dactylorhiza hatagirea* in the highest dose tested (400 mg/kg), shows an increase in Gastric pH and Gastric juice pH, whereas a decrease in Gastric content, Gastric juice volume, and Total acidity. Therefore, as per histopathological evaluation studies, it was concluded that *Dactylorhiza hatagirea*, at the highest dose of 400 mg/kg, was found to be safe and more effective in eradicating gastric ulceration. In Conclusion, based on the results obtained, the chloroform extract of *Dactylorhiza hatagirea-treated groups demonstrates a significant impact when compared with the control group animals, showing that the plant has anti-ulcer* activity. The antiulcer action of *Dactylorhiza hatagirea* was assessed by pylorus ligation-induced ulcer models. These models cause gastric ulcers in people. Numerous variables and instruments are associated with ulcerogenesis and gastric mucosal harm. Pylorus ligation-induced ulcer was utilized to note the impact of *Dactylorhiza hatagirea* extract on gastric acid secretion and bodily fluid emission. The ligature of the opening of the abdomen causes the accumulation of internal organ acid within the abdomen. This increase in the internal organ acid secretion causes ulcers within the abdomen. Ligation of the pyloric end of the stomach is made in 24 h fasted rats; the UI is resolved 4 h after pylorus ligation. The lesions created by this methodology are placed inside the lumen of the abdomen.

Keywords: Peptic Ulcer, *Dactylorhiza hatagirea*, Aspirin-Induced Ulcer, *H. pylori*, Hydroalcoholic Extract

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1. Introduction

Peptic ulcer, also known as *ulcus pepticum*, peptic ulcer disease (PUD), is an ulcer (defined as mucosal erosions equal to or greater than 0.5 cm of in area of the gastrointestinal tract) that is usually acidic and thus extremely painful [1-2]. As many as 80% of ulcers are associated with *Helicobacter pylori*, a spiral-shaped bacterium that lives in the acidic environment of the stomach, however, only 40% of those cases go to a doctor. Ulcers can also be caused or worsened by drugs such as aspirin and other NSAIDs [3-4].

An eroded lesion in the gastric intestinal mucosa is called a peptic ulcer. An ulcer may form in any part of the digestive tract, which is exposed to acidic gastric juice, but is usually found in the stomach and the duodenum [5]. The ulcer located in the stomach is known as a gastric ulcer, and that located in the duodenum is called a duodenal ulcer. Usually, both are grouped and termed peptic ulcer [6-7].

Peptic ulcer nowadays is a very common disease in the world. PUD encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder [8]. The pathophysiology of PUD involves an imbalance between offensive (acid, pepsin, and *H. pylori)* and defensive factors

(mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) [9]. There is a balance in the stomach between the aggressive digestive capabilities of acid plus pepsin and mucosal barrier. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance [10]. Several factors are implicated in the pathogenesis of gastric ulcer. These include increased acid-pepsin secretion, impaired bicarbonate neutralization, impaired mucus secretion and precipitate lesions on the mucosal layer. An estimated 15,000 deaths occur each year as a consequence of peptic ulcer diseases [11].

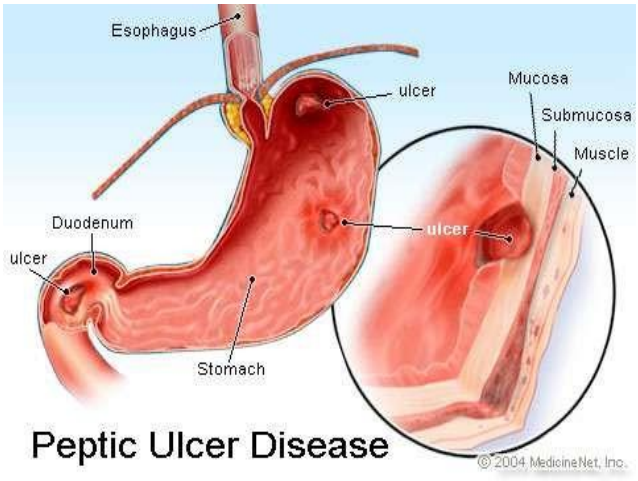

Figure 1: Showing site of Peptic ulcer

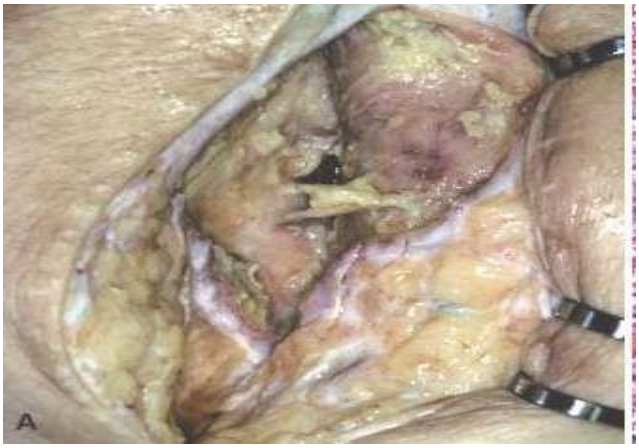

Figure 2: Peptic Ulcer

A major causative factor (60% of gastric and up to 90% of duodenal ulcers) is chronic inflammation due to *Helicobacter pylori* that colonizes the antral mucosa. The immune system is unable to clear the infection, despite the appearance of antibodies. Thus, the bacterium can cause a chronic active gastritis (type B gastritis), resulting in a defect in the regulation of gastrin production by that part of the stomach, and gastrin secretion can either be decreased (most cases) resulting in hypo- or achlorhydria increased. Gastrin stimulates the production of gastric acid by parietal cells and, in *H. pylori* colonization responses that increase gastrin, the increase in acid can contribute to the erosion of the mucosa and therefore ulcer formation [12].

Another major cause is the use of NSAIDs . The gastric mucosa protects itself from gastric acid with a layer of mucus, the secretion of which is stimulated by certain prostaglandins. NSAIDs block the function of cyclooxygenase 1 (cox-1), which is essential for the production of these prostaglandins. cox-2 selective anti-inflammatories (such as celecoxib or the since withdrawn rofecoxib) preferentially inhibit cox-2, which is less essential in the gastric mucosa, and roughly have the risk of NSAID-related gastric ulceration [13]. As the prevalence of *H. pylori*-caused ulceration declines in the Western world due to increased medical treatment, a greater proportion of ulcers will be due to increasing NSAID use among individuals with pain syndromes as well as the growth of aging populations that develop arthritis [14].

The incidence of duodenal ulcers has dropped significantly during the last 30 years, while the incidence of gastric ulcers has shown a small increase, mainly caused by the widespread use of NSAIDs [15]. The drop-in incidence is considered to be cohort- phenomena independent of the progress in treatment of the disease. The cohort-phenomena is probably explained by improved standards of living which has lowered the incidence of *H. pylori* infections.

Although some studies have found correlations between smoking and ulcer formation, others have been more specific in exploring the risks involved and have found that smoking by itself may not be much of a risk factor unless associated with *H. pylori* infection. Some suggested risk factors such as diet, spice, consumption and blood type, were hypothesized as ulcerogens (helping cause ulcers) until late in the 20th century, but have been shown to be of relatively minor importance in the development of peptic ulcers. Similarly, while studies have found that alcohol consumption increases risk when associated with *H. pylori* infection, it does not seem to independently increase risk, and even when coupled with *H. pylori* infection, the increase is modest in comparison to the primary risk factor.

Gastrinomas (Zollinger-Ellison syndrome), rare gastrin-secreting tumors, also cause multiple and difficult-to-heal ulcers.

Other causes of ulcers are conditions that can result in direct damage to the wall of the stomach or duodenum, such as heavy use of alcohol, radiation therapy, burns, and physical injury.

2. Materials And Methods

2.1 Plant Material Collection

Flowers of *Dactylorhiza hatagirea* were collected from local area of Bhopal (M.P.) in the month of January, 2024.

2.2 Extraction Procedure

The following procedure was adopted for the preparation of extract from the shade dried and powdered herbs.

2.2.1 Defatting of Plant Material

Powdered flowers of *Dactylorhiza hatagirea* were shade dried at room temperature. The shade dried plants material was coarsely powdered and preserved in an air tight bottle for further use, subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place [16].

2.2.2 Extraction by Maceration Process

50 g of dried plant material were exhaustively extracted with a hydroalcoholic solvent (80:20 ethanol: water) using the maceration method. The extract was evaporated above its boiling point. Finally, the percentage yields of the dried extracts were calculated [17].

2.2.3 Determination of Percentage Yield

Calculation of percentage yield

The percentage yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

2.3 Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

2.3.1 Detection of alkaloids:

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

2.3.2 Detection of carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

2.3.3 Detection of glycosides:

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

2.4 In vivo Anti-ulcer activity of Hydroalcoholic flower extract of *Dactylorhiza hatagirea (HEDH)*

2.4.1 Animals: -

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

2.4.2 Drugs & Chemicals

Ranitidine (Sigma Lab, Mumbai) were used in present study.

2.4.3 Toxicity study

Preliminary experiments were carried out on rats (n=6). Hydroalcoholic flower extract of *Dactylorhiza hatagirea* (HEDH) were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organization for Economic Co-operation and Development (OECD). Animals were kept fasting providing only water. HEDH were given p. o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-ulcer effect.

2.4.4 Experimental designs

Aspirin-induced gastric ulcer

Group –1: Control
Group –2: Ranitidine (Standard)
Group –3: HEDH (100 mg/kg, p.o.)
Group –4: HEDH (200 mg/kg, p.o.)

The animals were fasted for 24 h prior to the experiment. Under anesthesia, ulcers were induced by applying aspirin (500 mg/kg. p.o.) over the anterior serosal surface of the stomach for 60 seconds. The animals were treated with Ranitidine (50 mg/kg, p.o.), low dose of HEDH (100 m/kg p.o.) or high dose of HEDH (100 m/kg p.o.) [once daily, for 5 days after the induction of ulcer, while the control group received only the vehicle. The rats were sacrificed on the 5th day, the stomachs removed and cut open along the greater curvature. The ulcer index was determined using the formula:

Ulcer index = 10/X

Where X = Total mucosal area/Total ulcerated area.

Based on their intensity, the ulcers were given scores as follows:

0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, 3 = perforated or penetrated ulcer.

3. Results And Discussion

3.1 Result of the percentage yield of the extract

The yield of extracts obtained from the sample using the Hydroalcoholic solvent is depicted in Table 1

Table 1: Result of Percentage yield of Flowers of *Dactylorhiza hatagirea*

S. No.	Solvents	Percentage Yield
1.	Hydroalcoholic	8.12

3.2 Phytochemical analysis

The phytochemical analysis of HEDH was analysed (Table-2) for the compounds such as alkaloids, flavonoids, and glycosides, carbohydrates, saponins, phenols, proteins and amino acids and diterpenes. The preliminary phytochemical analysis revealed the presence of four compounds i.e. alkaloids, flavonoids, phenolics, saponins, and absence of glycosides, diterpenes, carbohydrate, proteins and amino acids. Various tests have been performed to find out the phytochemical constituents mentioned above [18].

Table 2: Result of Phytochemical Screening of HEDH

S. No.	Constituents	Hydroalcoholic Extract
1.	Alkaloids	+
2.	Glycosides	-
3.	Flavonoids	+
4.	Saponins	+
5.	Phenolics	+
6.	Amino Acids	-
7.	Carbohydrate	-
8.	Proteins	-
9.	Diterpenes	-

3.3 Results of estimation of total flavonoid and total phenol content of *Dactylorhiza hatagirea*

Natural antioxidants derived from plants, chiefly phenolics, are of considerable interest as dietary supplements or food preservatives. Hence, an attempt was made to quantify some secondary metabolites of HEDH. The total phenolic and flavonoid contents were analyzed and presented in table 3-5.

3.3.1 Total flavonoids content estimation (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **Y=0.040X + 0.009, R^2=0.999**, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 3: Preparation of Calibration Curve of Quercetin

S. No.	Concentration (µg/ml)	Absorbance
1	5	0.216
2	10	0.425
3	15	0.625
4	20	0.815
5	25	1.021

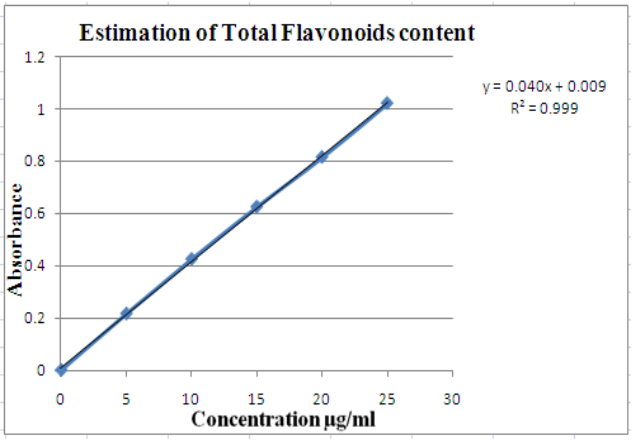

Figure 3: Graph of Estimation of Total Flavonoids Content

3.3.2 Total Phenolic content estimation (TPC)

The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.042X+0.002, R^2= 0.999,** where X is the gallic acid equivalent (GAE) and Y is the absorbance.

3.3.3 Calibration Curve of Gallic acid

Table 4: Preparation of Calibration Curve of Gallic acid

S. No.	Concentration	Absorbance
1	5	0.194
2	10	0.422
3	15	0.637
4	20	0.848
5	25	1.035

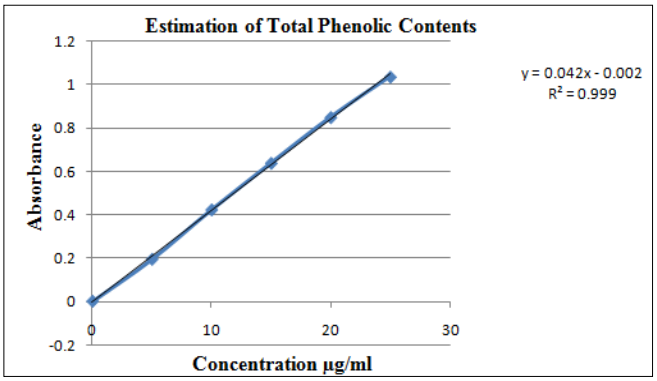

Figure 4: Graph of Estimation of Total Phenolic content

Table 5: Total Phenolic and Total Flavonoid Content of HEDH

S. No.	Solvents→ Bioactive compound↓	Hydroalcoholic extract
Flowers of *Dactylorhiza hatagirea*		
1.	Total Phenol (Gallic acid equivalent (GAE) mg/100mg)	1.039
2.	Total flavonoid (Quercetin equivalent (QE) mg/100mg)	0.941

Table 6: Anti-ulcerogenic effect of HEDH against ulcerogenic agents in rats (Ulcer index)

Treatment and dose	Aspirin
Control	3.80 ± 8.0
Ranitidine (50 mg/kg, p.o.)	1.80 ± 8.0***
HEDH (100 mg/kg, p.o.)	2.15 ± 8.0**
HEDH (200 mg/kg, p.o.)	1.76 ±8.0***

Values are expressed as mean±S.E.M. (n = 6).
Percent inhibition calculated as compared to control group. ****P* < 0.001, ** *P* < 0.01, * *P* < 0.05 (One-way ANOVA followed by Tukey's post hoc test).

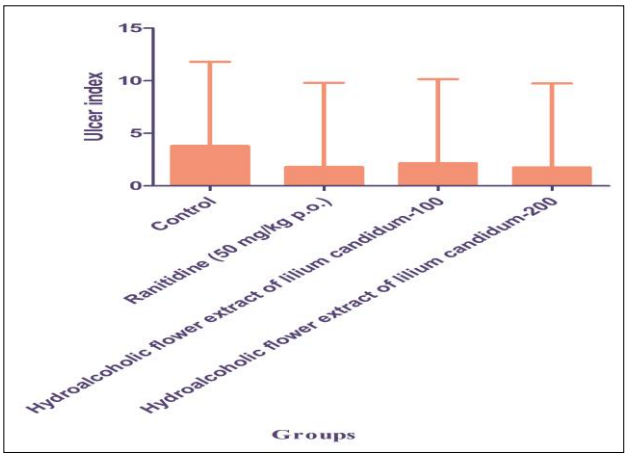

Figure 5: Anti-ulcerogenic effect of HEDH against ulcerogenic agents in rats (Ulcer index)

Table 7: Anti-ulcerogenic effect of HEDH against ulcerogenic agents in rats (PH)

Treatment and dose	Aspirin
Control	1.20 ± 5.0
Ranitidine (50 mg/kg, p.o.)	6.40 ± 5.0***
HEDH (100 mg/kg, p.o.)	5.20 ± 5.0*
HEDH (200 mg/kg, p.o.)	6.10 ± 5.0***

Values are expressed as mean±S.E.M. (n = 6).
Percent inhibition calculated as compared to control group. ****P* < 0.001, ** *P* < 0.01, * *P* < 0.05 (One-way ANOVA followed by Tukey's post hoc test).

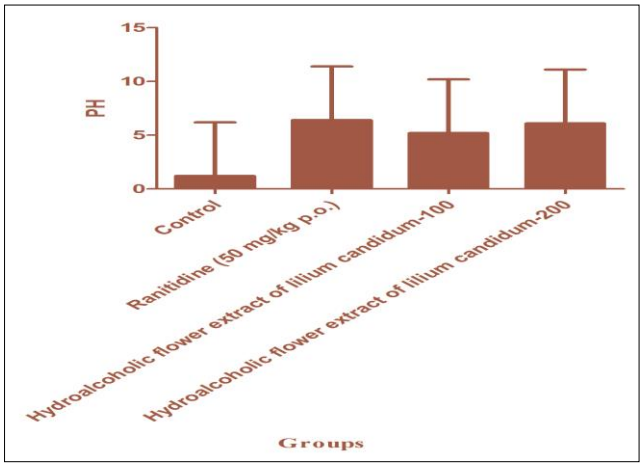

Figure 6: Anti-ulcerogenic effect of HEDH against ulcerogenic agents in rats (PH)

4. Discussion

The present study investigated the effect of HEDH on the ulcers. HEDH showed effect on the healing of gastric ulcers induced by aspirin. HEDH showed significant protection against aspirin-induced gastric ulcer in all dose levels. There is a dose-dependent increase in anti-ulcer effect of HEDH. HEDH was effective in reducing the ulcer area and the ulcer score.

5. Conclusion

The present study revealed that HEDH has ulcer-protective properties. Therefore, it can be considered as an ideal substitute for conventional NSAIDs and glucocorticoids. Further studies have to be conducted to explain precisely the mechanism of action of this drug. HEDH has an antiulcer effect. It increased healing of indomethacin induced ulcer.

References

[1]. Davidson-Hunt I.2000: Ecological ethno botany: stumbling toward new practices and paradigms. MASA J. ,16:1–13,2000

[2]. UNESCO. Culture and Health, Orientation Texts – World Decade for Cultural Development 1988 – 1997, Document CLT/DEC/PRO – 1996, Paris, France, pgs. 129,1996.

[3]. UNESCO. FIT/504-RAF-48 Terminal Report: Promotion of Ethno botany and the Sustainable Use of Plant Resources in Africa, pgs. 60, Paris, 1998.

[4]. Lucy Hoareau and Edgar J. DaSilva,: Medicinal plants: a reemerging health aid, Division of Life Sciences UNESCO

[5]. Lemma, A. The Potentials and Challenges of Endod, the Ethiopian Soapberry Plant for Control of Schistosomiasis. In: Science in Africa: Achievements and Prospects, American Association for the Advancement of Sciences (AAAS), Washington, D.C., USA, 1991.

[6]. Bassam Abdul Rasool Hassan. Medicinal Plants (Importance and Uses). Clinical Pharmacy Discipline, School of Pharmaceutical Sciences, University of Sains Malaysia, 11800, Minden, Penang, Malaysia, Pharmaceutica Analytica Acta, 2012

[7]. Encyclopedia of Ayurvedic Medicinal Plants: A Candle of Medicinal Herb's Identification and Usage.

[8]. Dixit, S Huma Ali. Antioxidant Potential Some Medicinal Plants of Central India, Journal of Cancer Therapy, 2010, 1, 87-90 Doi:10.4236/jct.2010.12014 Published Online June 2010 .

[9]. Nwankwo, J.O., 2011. Potential Anti - cancer and Antiviral Agents from West African Phytochemicals. University of Nigeria Press. Pp 156 162.

[10]. WHO.1991b. Traditional Medicine and Modern Health Care. WHO Geneva.

[11]. World Health Organization (WHO). National Policy on Traditional Medicine and Regulation of Herbal Medicines. Geneva: 2005. Report of WHO global survey.

[12]. Xutian S, Zhang J, Louise W. New exploration and understanding of traditional Chinese medicine. Am J Chin Med. 2009; 37:411–26.

[13]. Engebretson J. Culture and complementary therapies. Complement Ther Nurs Midwifery. 2002; 8:177–84.

[14]. Xutian S, Zhang J, Louise W. New exploration and understanding of traditional Chinese medicine. Am J Chin Med. 2009;37:411–26.

[15]. Barnes P. M, Bloom B, Nahin R. Complementary and alternative medicine use among adults and children: United States, 2007. CDC National Health Statistics Report # 12. 2008.

[16]. Cohen P. A, Ernst E. Safety of herbal supplements: A guide for cardiologists. Cardiovasc Ther. 2010; 28:246–53.

[17]. Engebretson J. Culture and complementary therapies. Complement Ther Nurs Midwifery. 2002; 8:177–84.

[18]. Eisenberg D. M, Davis R. B, Ettner S. L, Appel S, Wilkey S, Van Rompay M, Kessler R. C. Trends in alternative medicine use in the United States, 1990-1997: Results of a follow-up national survey. JAMA. 1998; 280:1569–75.