ANTI-ULCER POTENTIAL CHLOROFORM EXTRACT OF CUCUMIS SATIVUS

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ABSTRACT:

Ulcers are lesions on the surface of the skin or a mucous membrane characterized by a superficial loss of tissue. Helicobacter pylori is uniquely adapted to survival in the hostile environment of stomach. C. sativus is hairy and the root system is extensive and superficial. Leaves are alternate and simple. Flowers are yellow regular and unisexual. The matured fruit is about 30 cm long, roughly cylindrical and often slightly curved. The dried fruit of Cucumis sativus were collected from local area of Alwar, Rajasthan. Dried fruit of C. sativus were cut in to small pieces and dried under the shade. Water and alcoholic soluble extractive value of C. sativus was determined. 4.0 g fruits powder of C. sativus was weighed individually and macerated with distilled water (100 ml) in a glass-stoppered conical flask for 24 hours. Total ash, water soluble ash, acid insoluble ash and sulfated ash value of C. sativus was determined. 200 g coarse fruits powdered fruits were defatted with 800 ml petroleum ether (60-80°C) using soxhlet apparatus. Phytochemical screening of C. sativus extracts were carried out on the basis of qualitative chemical tests and TLC. Evaluation of anti-ulcer activity of C. sativus fruits by Acute toxicity method and ulcer index was calculated, the percentage yield of petroleum ether, chloroform and extract of C. sativus fruits was found to be 6.35 %, 7.26 % respectively. All these observation imply that the Chloroform extract of fruits of C. sativus could be regarded as a favourable antiulcerogen which could be attributed to its content of flavonoids and mucilage.

Keywords: Ulcers, Cucumis sativus, flavonoids, mucilage.

1. INTRODUCTION:

1.1 Ulcers:

Ulcers are lesions on the surface of the skin or a mucous membrane characterized by sloughing of inflamed dead tissue.¹ Ulcers are lesions on the surface of the skin or a mucous membrane characterized by a superficial loss of tissue. Ulcers are most common on the skin of the lower extremities and in the gastrointestinal tract, although they may be encountered at almost any site. Ulcers on the digestive tract membranes are called peptic ulcers (or stomach ulcers or duodenal ulcers).²

1.1.1 Peptic ulcer:

Peptic ulcer is defined as disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation.

Figure 1: Diagram of Peptic Ulcer

A peptic ulcer is a hole in the gut lining of the stomach, duodenum, or esophagus. A peptic ulcer of the stomach is called a gastric ulcer of the duodenum, a duodenal ulcer; and of the esophagus, an esophageal ulcer.
1.1.2 Types of Peptic Ulcer:

1.1.2.1 Gastric ulcer
1.1.2.2 Duodenal ulcer

1.2 Physiological factors in Gastric Ulcers:

Gastric ulcers almost invariably arise in the setting of H. pylori gastritis or chemical gastritis that results in injury to epithelium. Most patients with gastric ulcers secrete less acid than do those with duodenal ulcers and even less than normal persons. The factors implicated include:

a) Back-diffusion of acid into the mucosa.

b) Decreased parietal cell mass, and

c) Abnormalities of the parietal cells themselves.

Helicobacter Pylori (H. Pylori): Helicobacter pylori is a spiral-shaped Gramnegative bacterium that colonizes the stomach in about 50% of all humans. Helicobacter pylori is uniquely adapted to survival in the hostile environment of stomach. It attaches to the surface epithelium beneath the mucus, has high urease activity produces ammonia which maintains a neutral microenvironment around the bacteria and promotes back diffusion of H+ ions.

Non-steroidal anti-inflammatory drugs (NSAIDs): Ongoing use of this class of medications is the second most common cause of ulcers. These drugs (which include aspirin, ibuprofen, naproxen, diclofenac, tolmetin, piroxicam, fenoprofen, indomethacin, oxaprozin, ketoprofen, sulindac, nabumetone, etodolac and salicylates) are acidic and they block prostaglandins, substances in the stomach that help maintain blood flow and protect the area from injury. Some of the specific drugs listed are more likely to produce ulcers than others.27

2. Description of plant:

Botanical name: Cucumis sativus.
Common name: English- Cucumber, Hindi – Kheera, Sanskrit – Trapusa, Malayalam – Vellari.
Family: Cucurbitaceae.

Figure 2: Plant Cucumis sativus

2.1 Plant details:54

C. sativus is a creeping vine that roots in the ground and grows up trellises or other supporting frames, wrapping around supports with thin, spiraling tendrils. The plant is hairy and the root system is extensive and superficial. Leaves are alternate and simple. Flowers are yellow regular and unisexual. The matured fruit is about 30 cm long, roughly cylindrical and often slightly curved. The skin of the fruit is usually green, but in some cultivars white, brown or yellow is also available. This plant is originally from India but now is grown commercially all over the world. The fruit of this plant is often used for eaten fresh and pickling.
2. MATERIAL AND METHODS:

2.1 Collection and authentication of plant material
In the present study, the dried fruit of *Cucumis sativus* were collected from local area of Alwar, Rajasthan. The plant of *C sativus* has been authenticated from Botanical Survey of India, Jodhpur, Rajasthan, India. A voucher specimen has been deposited in the herbarium for future reference.

2.2 Drying of crude drugs material
Dried fruit of *C. sativus* were cut in to small pieces and dried under the shade. After the complete drying fruit were grinded to convert in to coarse powder for further studies.

2.3 Organoleptic characters of *C. sativus* powder
The following characteristic features were observed under the morphological studies.

2.4 Determination of physiochemical parameters of *C. sativus*
The coarse dried powder of *C. sativus* drugs were subjected to different standardization parameters as per WHO guideline.

2.4.1 Foreign matters
Medicinal plant materials should be entirely free from visible signs of contamination by moulds or insects, and other animal contamination, including animal excreta. No abnormal odour, discoloration, slime and signs of deterioration should be detected. Macroscopic examination can conveniently be employed for determining the presence of foreign matter in whole or cut plant materials.

**Procedure**
50 g coarse powder sample of *C. sativus* was weighed individually and spread over a white sheet of paper and foreign matter was sorted into groups by visual inspection by using a magnifying lens (10 X) and with the help of sieves. The sample sifted to the sieve no. 250, dust was regarded as material admixture. The portions of the sorted matter were weighed to within 0.05 g. The content of each group in grams per 100 g of air-dried sample was calculated.

2.5 Extractive values
Water and alcoholic soluble extractive value of *C. sativus* was determined by following procedure:

2.5.1 Water soluble extractive value

**Procedure**
4.0 g fruits powder of *C. sativus* was weighed individually and macerated with distilled water (100 ml) in a glass-stoppered conical flask for 24 hours. Shake frequently for 6 hours. Allowed to stand for 18 hours and then filtered rapidly. 25 ml of the filtrate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. It was dried at 105°C for 6 hours, cooled in a desiccators for 30 minutes and weighed without delay. The content of extractable matter was calculated in mg per g of air-dried material.

2.5.2 Chloroform soluble extractive value

**Procedure**
10.0 g powder of *C. sativus* was weighed individually and macerated with Chloroform (100 ml) in a glass-stoppered conical flask for 24 hours. Shake frequently for 6 hours. Allowed to stand for 18 hours and then filtered rapidly. 25 ml of the filtrate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. It was dried at 105°C for 6 hours, cooled in a desiccators for 30 minutes and weighed without delay. The content of extractable matter was calculated in mg per g air-dried material.

2.6 Ash value
Total ash, water soluble ash, acid insoluble ash and sulfated ash value of *C. sativus* was determined, following the WHO guideline standard procedure.

2.7 Loss on drying
Loss on drying method used to determine the presence of moisture content within the drug material.

**Procedure**
2.0 g leave powder of *C. sativus* was weighed individually in a tarred china dish and the initial weight was taken. The crude drug was heated at 105 ± 1°C in an oven and weighed. This procedure was repeated till a constant weight was obtained. The moisture content of the sample was calculated as percentage with reference to the dried material. Loss on drying of *C. sativus* is shown in Table 07.
2.8 Foaming index

Procedure
1.0 g leaf powder of C. sativus was weighed individually and transferred to a 500 ml conical flask containing 100 ml of boiling water, maintained at moderate boiling for 30 minutes. Cooled and filtered into a 100 ml volumetric flask and added sufficient water through the filter to dilute to volume. Poured the decoction into 10 stoppered test-tubes (height 16 cm, diameter 16 mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and adjusted the volume of the liquid in each test tube with water up to 10 ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second. Allow to stand for 15 minutes and measure the height of the foam.

Foaming index = \( \frac{1000}{a} \)

a = Volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

2.9 Extraction of C. sativus

2.9.1 Defattation of powdered C. sativus fruits

200 g coarse fruits powdered fruits were defatted with 800 ml petroleum ether (60-80°C) using soxhlet apparatus. Extraction was continued until a drop of solvent from siphon tube, when evaporated on filter paper, did not leave a greasy spot (approximately 10-12 cycles). After the defattation, mark was taken out from extractor and spreaded as a bed on a clean paper and dried till evaporation of petroleum ether. Mark was kept for ethanolic extraction. The light brown colored petroleum ether extract was collected and kept for phytochemical analysis.

2.9.2 Chloroform extraction of C. sativus fruits

The dried mark obtained after defattation was packed in soxhlet apparatus and extracted with 800 ml Chloroform in soxhlet apparatus. Extraction was continued until a drop of solvent from the siphon tube, when taken on TLC plate and sprayed with concentrated sulphuric acid, does not give a black spot. Dark brown extract thus obtained, was collected and solvent was evaporated under reduced pressure. Percentage yield of Chloroform extract was calculated.

The Chloroform extract and different fraction were stored for phytochemical analysis, isolation and pharmacological screening. Percentage yield, consistancy and color of different fraction of C. sativus fruits are shown in table 08.

2.10 Phytochemical screening of C. sativus extracts

Phytochemical screening of C. sativus extracts were carried out on the basis of qualitative chemical tests and TLC (thin layer chromatography).

2.11 Thin Layer Chromatography studies

Thin Layer Chromatography (TLC) was performed on silica gel G coated plates with a view to ascertain the number of components presents in the different extracts of C. sativus fruits.

Table 1: Thin layer chromatographic studies of ethanolic extract of C. sativus fruits

| Solvent systems | Spraying reagents |
|----------------|------------------|
| Flavonoids     |                  |
| Acetone:water (7:3) | 10% Alcoholic FeCl3 |
| n-Hexane:ethyl acetate(3:7) | 20% Alcoholic FeCl3 |
| Chloroform:ethyl acetate(3:7) | 20% Alcoholic lead acetate |
| Glycosides     |                  |
| Ethyl acetate: methanol:water (100:13.5:10) | Anisaldehyde sulphuric acid |
| Toluene:ethyl acetate:formic acid (2.5:1:1) | 10% Alcoholic KOH |
| Tannins        |                  |
| Toluene:acetone:formic acid (60:60:10) | Vanillin sulphuric acid |
| Aqueous FeCl3  |                  |
| Alkaloids      |                  |
| Toluene: ethyl acetate:diethylamine | Dragendorff's reagent |

2.12 Evaluation of anti-ulcer activity of C. sativus fruits

2.12.1 Acute toxicity method (Acute toxic class method)

The method enables a judgment with respect to classifying the test substances to one of the series of toxicity classes defined by fixed LD₅₀ cut off values. Acute toxicity describes the adverse effects of a substance which result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of the substance. Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at
lower levels, to a substance over a longer time period (months or years).

2.13 Ulcer Index

Procedure:

Animals in the group of ethanol induced ulcer were starved for 36 hours having access to drinking water ad libitum. 1ml of 80% ethanol would be administered orally. Famotidine is given to one group and Pedalium murex juice to the other groups 1 hour before the administration of ethanol. After 2 hours of ethanol administration, animals will be sacrificed by overdose of ether. The stomach was removed and fixed on a cork plate and the number of and severity of ulcers was registered with a stereo microscope using the following scores:

Calculation:

Ulcer index was calculated as;

\[ UI = UN + US \times 10^{-1} \]

Where:

* UI = ulcer index
* UN = average of number of ulcers per animal
* US = average of severity score
* UP = percentage of animals with ulcer

3. RESULT AND DISCUSSION:

3.1 Phytochemical evaluation:

| Phytoconstituents | Petroleum ether extract | Chloroform extract |
|------------------|-------------------------|--------------------|
| Steroid          | +                       | -                  |
| Triterpnonoids   | -                       | -                  |
| Glycosides       | -                       | +                  |
| Carbohydrates    | -                       | -                  |
| Alkaloids        | -                       | +                  |
| Flavonoids       | -                       | +                  |
| Tannins          | -                       | -                  |
| Proteins and amino acid | - | - |
| Lipids           | +                       | -                  |

Petroleum ether extract fruits showed presence of steroid, chloroform extract showed the presence of glycoside, alkaloid, flavanoid.

Table 6: T.L.C plate of C. sativus extracts under visible light

| S.no. | Phytoconstituents | Numbers of spot | Rf Value |
|-------|------------------|-----------------|---------|
| 1     | Flavonoids       | 1               | 0.75    |
| 2     | Glycoside        | 1               | 0.48    |
| 3     | Tannin           | 2               | 0.76    |
| 4     | Alkaloid         | 2               | 0.11, 0.43 |

3.2 Pharmacological evaluation:

3.2.1 Acute toxicity studies

Animals were observed at regular time intervals at least once during the first 30 minutes of initial dosing during the first 24 hrs. In all the cases no
death were observed in treated groups with in first 24 hrs. Additional observations like behavioral changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behavior pattern were observed in each group of rats. Attention was also given to observation of tremors and convulsions.

**Table 7: Acute toxicity study of petroleum ether extracts of *C. sativus***

| S.no | Dose (mg/kg b. wt) | Numbers of animals | Observation          |
|------|--------------------|--------------------|---------------------|
| 1    | 5                  | 3                  | All animals survived|
| 2    | 50                 | 3                  | All animals survived|
| 3    | 200                | 3                  | All animals survived|
| 4    | 300                | 3                  | All animals survived|
| 5    | 500                | 3                  | All animals survived|
| 6    | 1000               | 3                  | All animals survived|
| 7    | 1500               | 3                  | All animals survived|
| 8    | 2000               | 3                  | All animals survived|
| 9    | 5000               | 3                  | All animals survived|

**Table 8: Acute toxicity study of chloroform extracts of *C. sativus***

| S.no | Dose (mg/kg b. wt) | Numbers of animals | Observation          |
|------|--------------------|--------------------|---------------------|
| 1    | 5                  | 3                  | All animals survived|
| 2    | 50                 | 3                  | All animals survived|
| 3    | 200                | 3                  | All animals survived|
| 4    | 300                | 3                  | All animals survived|
| 5    | 500                | 3                  | All animals survived|
| 6    | 1000               | 3                  | All animals survived|
| 7    | 1500               | 3                  | All animals survived|
| 8    | 2000               | 3                  | All animals survived|
| 9    | 5000               | 3                  | All animals survived|

**Table 9: Acute toxicity study of hydroalchoolic extracts of *C. sativus***

| S.no | Dose (mg/kg b. wt) | Numbers of animals | Observation          |
|------|--------------------|--------------------|---------------------|
| 1    | 5                  | 3                  | All animals survived|
| 2    | 50                 | 3                  | All animals survived|
| 3    | 200                | 3                  | All animals survived|
| 4    | 300                | 3                  | All animals survived|
| 5    | 500                | 3                  | All animals survived|
| 6    | 1000               | 3                  | All animals survived|
| 7    | 1500               | 3                  | All animals survived|
| 8    | 2000               | 3                  | All animals survived|
| 9    | 5000               | 3                  | All animals survived|

**Table 10: Dose dependent studies of the fresh extract of fruits of *C. sativus* using rats in ethanol induced ulcer model.**

| S.N. | Treatment          | No of Animals | Dose       | Ulcer Index | Total Acidity (mEq/L) | Acid Volume (ml) | pH       |
|------|--------------------|---------------|------------|-------------|-----------------------|------------------|----------|
| 1    | Control (water)    | 6             | ---        | 10.14±0.32  | 117.1±1.12            | 8.31±0.24        | 2.3±0.12 |
| 2    | Famotidine         | 6             | 0.3ml/100gm| 4.23±0.32** | 55.6±0.54**           | 4.65±0.31**      | 4.94±0.12** |
| 3    | FEFCS              | 6             | 0.5ml/100gm| 8.76±0.21   | 95.5±0.22*            | 6.55±0.13*       | 3.22±0.27 |
| 4    | FEFCS              | 6             | 1.0ml/100gm| 6.19±0.31*  | 75.0±0.56*            | 5.23±0.19*       | 3.81±1.17** |
| 5    | FEFCS              | 6             | 2.0ml/100gm| 1.45±0.21** | 62.3±0.31**           | 5.03±0.07**      | 4.13±0.53** |

FJLPM - fresh juice of leaves of *C. sativus* **P<0.001, *P<0.05, compared with control.**

**Figure 1:** Dose dependent studies of the fresh extract of fruits of *C. sativus* using rats in ethanol induced ulcer model.
4. CONCLUSION:

The present study has been done to evaluate the antulcer effect of Chloroform extract of fruits of C. sativus on ethanol induced ulcer models at various doses and time intervals. The results obtained from the present study have shown that Chloroform extract of fruits of C. sativus possesses antulcer effect on ethanol induced ulcers. Pretreatment with FEFCS particularly at a dose of 2.0ml/100gm in a single schedule and 1.0ml/100gm for 15 and 30 days treatment decreases ulcer index, total acidity, total volume of acid secretion and total protein and increase in pH and glutathione content when compared with control. All these observation imply that the Chloroform extract of fruits of C. sativus could be regarded as a favourable antiulcerogen which could be attributed to its content of flavonoids and mucilage.

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