ISSN: 2279 - 0594

Journal of Biomedical and Pharmaceutical Research

Available Online at www.jbpr.in

CODEN: - JBPRAU (Source: - American Chemical Society)

Index Copernicus Value: 72.80

PubMed (National Library of Medicine): ID: (101671502)

Volume 7, Issue 1: January-February: 2018, 47-54

Research Article

EVALUATION OF ANTI-ULCER ACTIVITY OF CHLOROFORM AND ETHER EXTRACT OF *JASMINUM OFFICINALE* L. LEAF

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Received 09 Dec. 2017; Accepted 20 Jan. 2018

ABSTRACT

*Jasminum Officinale*L. leaf had been collected form herbal garden of dravyagunavigyan, National Institute of Ayurveda and Botanical Authenticate Botanist of Institute of Biomedical and Industrial Research, the microscopical sections of the specimen were made and examined microscopically. Moisture content was determined by placing weighed sample of 5gm of drug in oven at 105º for 5 hours, The pH value of an aqueous liquid measured by using digital pH meter. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug. Proceed as directed for the determination of alcohol-soluble extractive, using distilledwater instead of ethanol, Determination of Petroleum Ether Soluble Extractive (Fixed Oil Content,The total ash method is design to measure the total amount of material remaining after ignition, Water – soluble ash value determined as per Pharmacopoeia of India 1996, Freshly prepared extracts were tested for the presence of various active phytocompounds like phenols, tannin, flavonoid, protein, reducing sugar, carbohydrates, lipids, saponin, triterpenoid alkaloid, resins, volatile oils, anthraquinone and Quinone. TLCTest sample*Chloroform extract of Leaf JasminumOfficinale Petroleum Ether extract of Leaf JasminumOfficinale*Tolune:Ethylacetate:Formic acid (6:3:1) USED AS mobile solution, extract prepare byFresh leaves were collected, shade-dried and powdered mechanically. About 60 g of the leaf powder were extracted with 600 ml of chloroform and petrolium ether each by maceration at room temperature for 7 days. The extract was obtained by vacuum distillation and dried at 40º C and the yield of the chloroform extract and petrolium ether extract was 26% and 22% respectively. acute toxicity study determined byRats were kept overnight fasting prior to drug administration. Six animals for each extract *Jasminumofficinale*L. leaf chloroform extract (JOL-CE) and ether extract (JOL-EE)] were used which received a single oral dose (2000 mg/kg, body weight for each extract). After the administration of JOL-CE and JOL-EE, food was withheld for further 3–4 h. EVALUATED BY Acid secretory parameters like gastric volume, pH, free and total acidity &Ulcer Index,Histopathological evaluation of ulcers**.** aspirin induced ulcer in rats were expressed as mean ± S.E.M. Data were analysed by two way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test using Graph pad Prism 7 software, with the level of significance set at $P < 0.05$ (at 95 % confidence interval).

Keywords: Jasmine, Helicobacter pylori, *JasminumOfficinale*, Anti-Ulcer

INTRODUCTION:

Peptic ulcer disease is a group of disorders characterized by the presence of ulcers in any portion of gastrointestinal tract (GIT) exposed to acid in sufficient concentration and duration. Although these ulcerations most commonly occur in the stomach (gastric ulcer), or small intestine (duodenal ulcer), this disease also includes Barrett ulcer of the esophagus (Barrett's esophagus or Barrett's metaplasia) and other upper GI ulcers.[1]

Acid secretion and peptic ulcers: The formation of peptic ulcers depend critically on the presence of acid and peptic activity in gastric juice. About one third of patients with duodenal ulcer, but not

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gastric ulcer, secrete excess gastric acid. Schwartz's dictum "no acid - no ulcer" is more accurate if amplified to "no acid and peptic activity - no ulcer" as acid without pepsin has little digestive power. The dependence of peptic activity is supported by the therapeutic effects of the antacids and antisecretory drugs (anticholinergics, H2blockers).[2]

Symptoms

Burning stomach pain

Feeling of fullness, bloating or belching

Fatty food intolerance

Heartburn

Nausea

Causes

Peptic ulcers occur when acid in the digestive tract eats away at the inner surface of the stomach or small intestine. The acid can create a painful open sore that may bleed.

Your digestive tract is coated with a mucous layer that normally protects against acid. But if the amount of acid is increased or the amount of mucus is decreased, you could develop an ulcer. Common causes include:

Regular use of certain pain relievers. Taking aspirin, as well as certain over-the-counter and prescription pain medications called nonsteroidal anti-inflammatory drugs (NSAIDs) can irritate or inflame the lining of your stomach and small intestine. These medications include ibuprofen (Advil, Motrin IB, others) and naproxen sodium (Aleve, Anaprox, others), but not acetaminophen (Tylenol)

Jasmine (taxonomic name Jasminum) is a genus of shrubs and vines in the olive family (Oleaceae). It contains around 200 species native to tropical and warm temperate regions of Eurasia, Australasia and Oceania. Jasmines are widely cultivated for the characteristic fragrance of their flowers.

Taxonomy of plants-[3]Kingdom Plantae – Plants
Subkingdom Tracheobionta – Vascular plants
SuperdivisionSpermatophyta – Seed plants
Division Magnoliophyta – Flowering plants
Class Magnoliopsida – Dicotyledons
Subclass Asteridae
Order Scrophulariales
Family Oleaceae – Olive family
Genus *Jasminum L.* – jasmine p

MATEIALS & METHOD:

Material

Test Sample:

*JasminumOfficinale*L. leaf had been collected form herbal garden of dravyagunavigyan, National Institute of Ayurveda and Botanical Authenticate Botanist of Institute of Biomedical and Industrial Research, Rajasthan

Glassware and Instruments:

Beaker, Taste Tube, Test tube stand and Holder, conical flask, volumetric flask, crucible, Petridis, Glass Slide, Coverslip, Microscope with Camera, Hot Air Oven, pH Meter, Soxhlet Assembly, Rotary shaker, TLC chamber.

Digital Weighing Balance, Microtome, Dissection box, Slides, Oral feeding needle and various glassware.

Table 1: List of Instruments

S. No.	Instrument name	Model
1.	Soxhlet apparatus	Borosilicate glass
2.	Water bath	Instrument, Esmail Building, Mumbai
3.	Incubator	Instrument, Esmail Building, Mumbai
4.	UV spectrophotometer	Shimadzu,1800
5.	Laboratory Centrifuge	Instrument, Esmail Building, Mumbai
6.	Heating mental	Instrument, Esmail Building, Mumbai

Table 2: List of chemicals

S. No.	Chemicals used	Company
1.	Acetic acid	CDH, New Delhi
2.	Acetone	RDH, Jaipur
3.	Chloroform	QFC, Mumbai
4.	Methanol	RDH, Jaipur
5.	Fehling's solution	CDH, New Delhi
6.	Sodium chloride	CDH, New Delhi
7.	Concentrated hydrochloric acid	RFCL, New Delhi
8.	Mayer's reagent	OI, Mumbai
9.	Concentrated sulphuric acid	RFCL, New Delhi
10.	Ethanol	JCPW, Jaipur
11.	Hager's reagent	CDH, New Delhi
12	Lead acetate solution	CDH, New Delhi
13.	Gelatin	EC, Mumbai
14.	Molisch's reagent	CDH, New Delhi
15.	Benedict's reagent	CDH, New Delhi
16.	Ferric chloride solution	CDH, New Delhi
17.	Conc. Nitric acid solution	Laboratory solution
18.	Bovine albumin	CDH, New Delhi
19.	Disodium hydrogen phosphate	CDH, New Delhi
20.	Potassium dihydrogen phosphate	CDH, New Delhi

Method:

Macroscopic:-Observe Shape, Colour, texture and visualized features.

Microscopy:-

Microscopic sections were cut by Microtome sectioning. Numerous temporary and permanent mounts of the microscopical sections of the specimen were made and examined microscopically. Histochemical reactions were applied with staining reagents on transverse sections and on bark powder by reported methods.

Determination of moisture content:-

Moisture content was determined by placing weighed sample of 5gm of drug in oven at 105º for 5 hours, and calculate weight of sample for every 30 minute, until the weight of the sample were constant, no variation of weight are recorded.

Determination of pH value:-

The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in gram per liter.

The pH of a given solution is measured by using digital pH meter.

Determination of Alcohol Soluble Extractive-[4]:-

It was taken Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allow to stand for eighteen hours.

Determination of Water Soluble Extractive:-

Proceed as directed for the determination of alcohol-soluble extractive, using distilled water instead of ethanol.

Determination of Petroleum Ether Soluble Extractive (Fixed Oil Content)[5]:-

Transferred a suitably weighed quantity (depending on the fixed oil content) of the air-dried, crushed drug to an extraction thimble, extract with solvent ether (or petroleum ether, b.p. 40º to 60º) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filtered the extract quantitatively into a tared evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105º to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

Determination of Total Ash:-Silica Crucible was cleaned, dried well, labeled with glass pencils and then weighed to constant weight. 5 gm of powdered drug sample put in the Silica crucible. The drug was spread evenly in to a thin layer. This crucible was placed in a muffle furnace and ignited at a temperature of 450°C for about 6 hrs or more until the ash was totally free from Carbon.

Determination of acid insoluble Ash:-

Acid insoluble Ash value determined as per Pharmacopoeia of India, 1996. Boiled the total ash (Prepared by method 2), with 25 ml of 2M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ashless filter paper, washed with hot water, ignite, cool in a desiccator and weighed. Calculated the percentage of acid - insoluble ash with reference to the air - dried drug.[7]

Chromatography plates-

T.L.C. plate coated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator, (Mercks) were used. (Each plate dimension is 10 cm long and 2 cm width)

Activation of pre-coated Silica gel G60F254

Dry in hot oven at 1050 C for one to two hour.

Test sample:

Chloroform extract of Leaf JasminumOfficinale

Petroleum Ether extract of Leaf Jasminum Officinale

Preparation of mobile solution

Tolune: Ethylacetate: Formic acid (6:3:1)

Sample application

Sample was applied with the help of capillary 1(one) cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1(one) cm below the top of the T.L.C. plate.[8]

Visualization:

Day light (Leaf of Stevia)

Rf. Value-

Measure and record the distance of each spot from the point of its application and calculate the Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.[9]

Plant collection

*Jasminumofficinale*leaves were collected from herbal garden of dravyagunavigyan, National Institute of Ayurveda (NIA), Jaipur. The plant was identified and authenticated by Botanist of BilwalMedchem and Research Laboratory Pvt. Lt., Siker, Rajasthan, India

Preparation of the extract

Fresh leaves were collected, shade-dried and powdered mechanically. About 60 g of the leaf powder were extracted with 600 ml of chloroform and petrolium ether each by maceration at room temperature for 7 days. The extract was obtained by vacuum distillation and dried at 40º C and the yield of the chloroform extract and petrolium ether extract was 26% and 22% respectively.[10]

Animals

Albino rats of Wistar strain of either sex weighing between 150 and 200 g were used. They were housed in standard cages at room temperature (25±2º C) and provided with food and water *ad libitum*. The animals were deprived of food for 24 h before experimentation, but had free access to drinking water. The study was conducted after obtaining institutional ethical committee clearance bearing the number 1737/PO/Rc/S/14/CPCSEA.[11]

Acute toxicity study

Rats were kept overnight fasting prior to drug administration. Six animals for each extract *Jasminumofficinale*L. leaf chloroform extract (JOL-

CE) and ether extract (JOL-EE)] were used which received a single oral dose (2000 mg/kg, body weight for each extract). After the administration of JOL-CE and JOL-EE, food was withheld for further 3–4 h.[12]

Aspirin induced ulcer in rats

Solutions of JOL-CE, JOL-EE, aspirin and standard antiulcer drug, ranitidine (Dharmani et al., 2005; Gupta et al., 2005) were prepared in 0.5% w/v sodium carboxy methyl cellulose (CMC) suspension as vehicle and administered orally once daily at a volume of 10 ml/kg body weight. The animals were divided into five groups, consisting of six rats in each group (having H, B, T, HB, BT, HT marking respectively).[13]

Evaluation:

a) Acid secretory parameters like gastric volume, pH, free and total acidity

The gastric contents were collected, centrifuged and the volume of the supernatant was expressed as ml/100 g body weight. Free and total acidity were determined by titrating with 0.01 N NaOH using Topfer's reagent (Dimethyl yellow in methanol) and phenolphthalein as indicator (Parmar et al., 1984). One ml of gastric juice is pipette into 100 ml conical flask, added 2 to 3 drops of topfer's reagent (Dimethyl yellow in methanol + phenolphthalein) and treated with 0.01 N sodium hydroxide until all traces of red colour disappears. The free and total acidity were expressed as Equiv./100 g/4 h. Acidity for each group were calculated by using the following formula- Acidity = (volume of NaOH × Normality of NaOH ×100)/0.1 m.eq/lit/100gms.

b) Ulcer index

The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass and the diameter of the ulcers were measured using vernier calipers. The following arbitrary scoring system (Dharmani et al., 2005) was used to grade the incidence and severity of lesions (Ulcer score)[14]

c) Histopathological evaluation of ulcers

Stomachs were immersed in a 10% formalin solution for histopathological examination following the assessment of ulcer score. The central part of the damaged (or) ulcerated tissue (if present) was cut in half along the long diameter.

Statistical analysis

Theexperimental results of aspirin induced ulcer in rats were expressed as mean ± S.E.M. Data were analysed by two way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test using Graph pad Prism 7 software, with the level of significance set at $P < 0.05$ (at 95 % confidence interval)[15]

RESULT & DISCUSSION:

Acute toxicity studies

In LD50 studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed. So as per the OECD guideline 423, 2002, the biological evaluation was carried out at doses of 200 mg/kg body weight.

Aspirin induced ulcer in rats

Animals in the Aspirin group showed a significant ($P < 0.05$) increase in the ulcer index and acid secretory parameters like gastric volume, pH, free and total acidity when compared with those of vehicle treated group. In the rats of this group, a number of perforated ulcers (score 3) were also observed.

Table 3: Effect of *Jasminumofficinale*L. leaf Chloroform extract (JOL-CE) or Ether extract (JOL-EE) on gastric secretion using aspirin induced ulcer in rats

Treatment	Dose (mg/kg)	Gastric volume	pH	Free acidity (µequiv/100 g/4 h)	Total acidity (µequiv/100 g/4 h)	Ulcer Index	% inhibition
Control	--	0.273 ± 0.0729	4.82 ± 0.367	11.1 ± 1.35	18.1 ± 1.57	2.31± 1.71	76.47
Aspirin	200 (p.o.)	2.61 ± 0.151	1.04 ± 0.0977	87.7 ± 1.9	105 ± 2.54	9.8± 0.526	---
JOL-CE	200 (p.o.)	0.61 ± 0.0266	3.13 ± 0.145	35.6 ± 1.4	42.5 ± 1.63	4.17± 1.47	57.47
JOL-EE	200 (p.o.)	0.583 ± 0.0297	3.48 ± 0.098	24.3 ± 0.714	39.3 ± 1.49	3.53±1.61	64.03
Ranitidine	20 (p.o.)	0.46 ± 0.0342	5.15 ± 0.238	14.6 ± 1.1	24.6 ± 1.21	2.83± 1.69	71.17

Data are expressed as the mean ± S.E.M.; n=6 in each group

Table 4: Two way ANOVA followed by Tukey's multiple comparisons test summary for the Effect of *Jasminumofficinale*leaf Chloroform extract (JOL-CE) or Ether extract (JOL-EE) on gastric secretion using aspirin induced ulcer in rats

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Difference is significant?	Summary	P Value
Control vs. Aspirin	-33.9	-52.7 to -15.1	Yes	***	<.001
Control vs. JOL-CE	-9.87	-28.7 to 8.95	No	ns	.595
Control vs. JOL-EE	-6.89	-25.7 to 11.9	No	ns	.849
Control vs. Ranitidine	-2.19	-21 to 16.6	No	ns	.998
Aspirin vs. JOL-CE	24.1	5.23 to 42.9	Yes	**	.005
Aspirin vs. JOL-EE	27	8.21 to 45.9	Yes	**	.001
Aspirin vs. Ranitidine	31.7	12.9 to 50.6	Yes	***	<.001
JOL-CE vs. JOL-EE	2.98	-15.8 to 21.8	No	ns	.992
JOL-CE vs. Ranitidine	7.68	-11.1 to 26.5	No	ns	.790
JOL-EE vs. Ranitidine	4.7	-14.1 to 23.5	No	ns	.958

No means there were no significant difference in the respective two groups i.e. both respective groups were approximately similar while Yes means there were significant difference in the respective two groups i.e. both respective groups were NOT similar, they had a significant value difference. In animal experimental data both statistical No and Yes data between different respective groups are needed.

Histopathology of aspirin-induced ulcers

After 10 days of treatment, the rats treated with aspirin showed loss of gland architecture with erosion of the epithelial layer and evident oedema and infiltration by inflammatory cell (Fig. 6). JOL-CE (200 mg/kg) treated rats showed no ulceration but intactness of gastric epithelium was not completely restored. Minimal oedema and infiltrationwas seen in the lower half of the mucosa (Fig. 7). JOL-EE (200 mg/kg) treated rats showed no ulceration in the mucosa. Glands are regular with complete restoration of gastric epithelium. Minimal oedema and infiltration were seen in one area (Fig. 8). Ranitidine treated groups showed no ulceration in gastric mucosa, glands were regular and no inflammation was observed (Fig. 19).

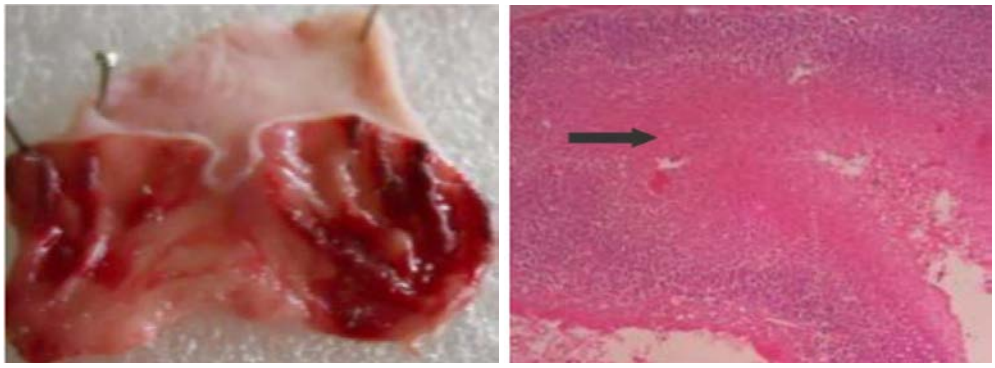

Figure 1: Section of ulcerated stomach obtained from rats of control group treated with aspirin-induced ulcer model in rats after 10 days of treatment (*n*=6 in each group). HE 100×.

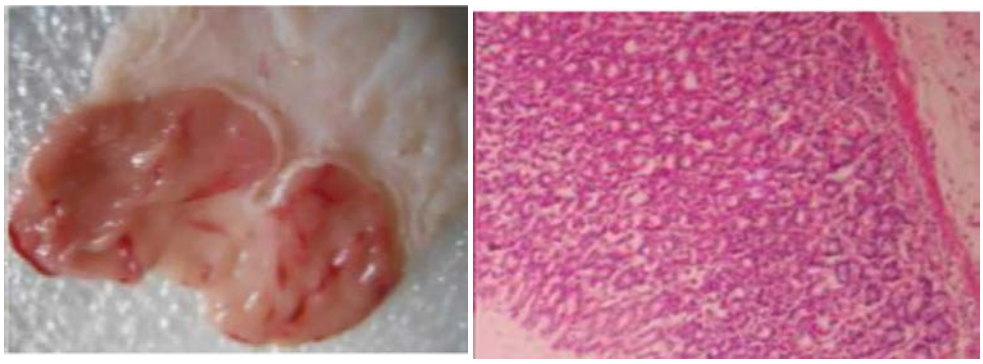

Figure 2: Section of ulcerated stomach obtained from rats of group treated with JOL-AE (200 mg/kg) in aspirin -induced ulcer model in rats after 10 days of treatment (*n* = 6 in each group). HE 100×.

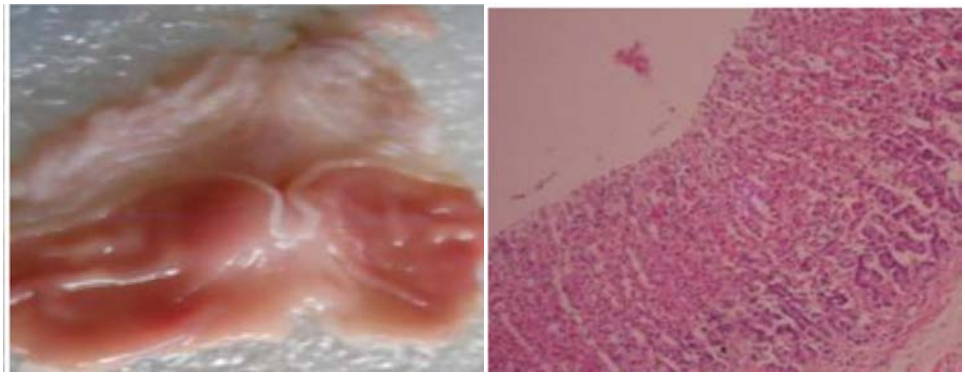

Figure 3: Section of ulcerated stomach obtained from rats of group treated with JOL-EE (200 mg/kg) in aspirin -induced ulcer model in rats after 10 days of treatment (*n* = 6 in each group). HE 100×.

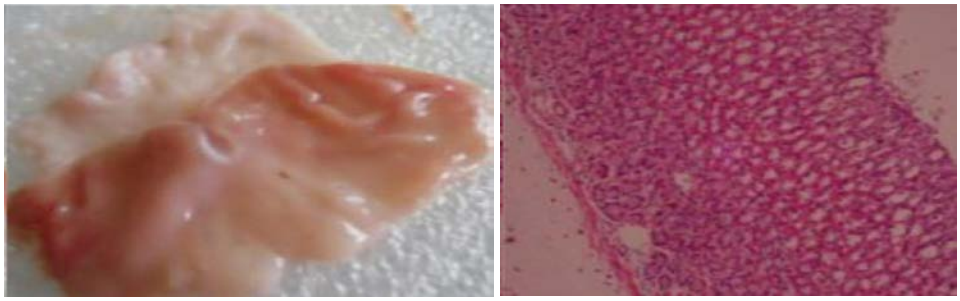

Figure 4: Section of ulcerated stomach obtained from rats of group treated with ranitidine (20 mg/kg) in aspirin -induced ulcer model in rats after 10 days of treatment (*n* = 6 in each group). HE 100×.

CONCLUSION:

The presented results offer supporting evidence for effective use of selected plant extracts for peptic ulcer activity. *Jasminum*officinale naturally possess variety of therapeutic agents but properties depend on the component of the plant, the system used to isolate these agents and method followed to evaluate the particular character. In the current study ether extract of *Jasminumofficinale*leaf exhibited comparatively better activities than chloroform extract in the assay seemingly due to efficient extraction of phytochemicals.

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