



SCREENING OF ANTI-PEPTIC ULCER ACTIVITY OF *JASMINUM SAMBAC*

JASMINUM SAMBAC'IN ANTI-PEPTİK ÜLSER AKTİVİTE TARAMASI

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ABSTRACT

Objective: The aim of this study was to investigate the antiulcer activity of callus derived metabolites.

Material and Method: The effect of different (2,4-Dichloro-Phenoxyacetic Acid(2,4-D), Indole-3-Acetic acid, (IAA), Indole-3-Butyric Acid(IBA), Naphthalene Acetic Acid(NAA)) growth hormones and their combination on callus formation, callus texture and weight (fresh and dry) of *Jasminum sambac* were investigated. In this investigation revealed that the best callus performance seen in individual MS medium containing 1.5 mg/L 2,4-D and combination 1.5 mg/L 2,4-D along with 1.5 mg/L IAA. Antiulcer activity was studied using the pylorus ligation method. A dosage of 200mg/kg b.w used for albino wistar rats was selected from the LD₅₀ study. Ulcer inhibition and ulcer score, gastric volume, pH, free acidity, total acidity and pepsin, carbohydrate and protein ratio were analyzed.

Result and Discussion: Plant has show higher activity in percentage of callus formation in MS media supplemented with single hormone of 1.5 mg/L 2,4-D. The friable callus was observed in single and combination of the hormone concentration. The callus extract also comprehensively decreased the gastric volume, free and total acidity, and improved the pH of the gastric fluid, proving its antisecretory activity. The results of this study showed that the surveyed extracts of the investigated callus exhibited potent antiulcerogenic activity and dose related activity.

Keywords: 2,4-D, callus, indole-3-acetic acid, *Jasminum sambac*, ulcer score

ÖZ

Amaç: Bu çalışmanın amacı, kallus kaynaklı metabolitlerin ülser önleyici aktivitesini araştırmaktır.

Gereç ve Yöntem: Farklı (2,4-D, IAA, IBA, NAA) büyüme hormonlarının ve bunların kombinasyonlarının kallus oluşumu, kallus dokusu ve *Jasminum sambac*'in ağırlığı (taze ve kuru) üzerindeki etkisi araştırılmıştır. Bu araştırmada, 1.5 mg/L 2,4-D ve 1.5 mg /L 2,4-D kombinasyonu ve 1.5 mg / L IAA içeren bireysel MS ortamında en iyi kallus performansının ortaya çıktığı görülmüştür. Antiülser aktivite pilorligasyonu yöntemi

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kullanılarak çalışılmıştır. Wistar albino sıçanlara uygulanan 200 mg/kg vücut ağırlığı dozu LD50 çalışmasından seçilmiştir. Ülser inhibisyonu, ülser skoru, gastrik hacim, pH, serbest asitlik, toplam asitlik ve pepsin, karbonhidrat ve protein oranı analiz edilmiştir.

Sonuç ve Tartışma: Bitki, 1.5 mg / L 2,4-D tek hormon ile takviye edilmiş MS ortamında kallus oluşumu yüzdesinde daha yüksek aktivite göstermiştir. Gevrek kallus, hormon konsantrasyonunun tekli ve kombinasyonunda gözlemlendi. Kallus ekstresi ayrıca mide hacmini, serbest ve toplam asiditeyi kapsamlı bir şekilde düşürdü ve mide sıvısının pH'ını iyileştirerek salgı önleyici aktivitesini kanıtladı. Bu çalışmanın sonuçları, araştırılan kallusun incelenen ekstrelerinin güçlü anti-ülserojenik aktivite ve doza bağlı aktivite sergilediğini gösterdi.

Anahtar Kelimeler: 2,4-D, indol-3-asetik asit, *Jasminum sambac*, kallus, ülser skoru

INTRODUCTION

Plants are the basis of sophisticated approaches to conventional medicine which have been used for millions of years and continue to manage mankind new remedies. *Jasminum sambac* commonly referred to as jasmine of the Oleaceae family of Indian origin. Jasmine is a major traditional flowering crop in India. These flowers are white and used to produce perfumes and aromatizers. The plant is used as aliana for neck and hair decoration in the form of a choker. Jasmine oil has broad range of therapeutic and cosmetic use including perfumery, soaps, flavourings [1]. Essential oil of this flowers has been used as aroma for skin care products, reduces anti inflammation, tones the skin and lifts up mood. Medicinally, it has been used for the action of dry, greasy, sensitive skin and irritated, irritating coughs, alleviating muscular pain, antiseptic, antidepressant, antispasmodic, treating sprains, sedative and uterine tonic. To manufacture high quality jasmine tea, the quality of jasmine flowers is critically important in addition to the quality of the tea leaves used as its foundation. Plant's used traditionally as an antidepressant, analgesic, antiseptic, anti-inflammatory, sedative, aphrodisiac, expectorant and tonic. [2].

Approximately 80 percentage (%) of the world population depend on the curative plants in the form of conventional for their primary health care. Biotechnology approaches, exclusively culture of plant tissue plays an important role in investigating for alternatives to plant based processing of medicinal compounds. Biotechnology offers to develop tissue, organs, cells or total organism by mounting them *in vitro*. It can able to produce required compound synthesis [3-5]. Utilize of methodologies of plant cell and organ culture as way to producing secondary metabolites has a long history.

The production of callus can be achieved from dissimilar vegetative organs, such as the root, leaf, stem, node, shoot tip, petiole, flower bud and embryo [6]. Immature vegetative organs are extra effective for the stimulation of callus. Explants selection and source is one of the significant parameters for successful term for cell culture. Callogenesis is also kind dependent relative on explants.

Gastric ulcer a form of failing narrow tissue associated with various causes is a disease common worldwide. It is habitually imitative from the submucous, mucosa, serosa layers and muscle of stomach,

which are soaked in the gastric juice, gastric acid, and pepsinum. Peptic ulcer is a largely common gastrointestinal disorder in experimental practice [7]. It is a defect in the liner of the stomach or the initial division of the duodenum. It becomes one of the community health problem with high morbidity and significant mortality and has become the focus of experimental and clinical investigations mainly owing to its high incidence in the general population.

Every year peptic ulcer affects almost four million individuals worldwide and affects 10% of global population with dissimilar aetiologies. Ulcers are open sore of the skin or mucus membrane categorized by sloughing of reddened dead tissue [8]. The objective of the present study was to investigate the antiulcer activity of the methanolic extract of *Jasminum sambac* callus using pylorus ligation model induced gastric peptic ulcer.

MATERIAL AND METHOD

Plant Collection and Identification of Plants

The plants used in this study *Jasminum sambac* (L.) Ait. (Figure. 1) No.BSU/SRC/5/23/2011-12/Tech-133 (Oleaceae), was collected from Namakkal District, Tamil Nadu. It was authenticated at Botanical Survey of India, South Circle, Coimbatore, Tamil Nadu. Herbarium is maintained in the Department of Biotechnology, Karpagam University, Coimbatore, TamilNadu.



Figure 1. *Jasminum sambac* (L.) Ait.

Medium and culture condition

MS (Murashige and Skoog) media was used for all over this study. Different hormone and combination of 2,4-D, IAA, IBA, NAA (0.5, 1.0, 1.5, 2.0 mg/L) used in this medium consist of 30 g/L sucrose and 8g/L agar and the pH was adjusted to be within 5.6-5.8. The media were autoclaved at 121°C under 15lbs for 20 minutes. About 1 cm sides of leaf square were selected as explants leaving short length of petiole attached from healthy plant. Explants sterilization and inoculation were done [9]. The sterilized explants were inoculated aseptically. All the cultures were maintained in the culture room temperature at 25±2°C at a humidity of 65-70% photo period of 16/8 h was maintained for all

experiments. Each experiment was repeated three times and percentage of callus formation was observed. Concurrently callus texture and weight (fresh and dry) were also calculated. The percentage of formation callus was recorded after 15 days of inoculation. The increase in mass as gain in weight was recorded as fresh weight of callus after 4 weeks of inoculation. The dried fresh callus weight was observed as dry weight.

Callus induction (%) = (Number of plants produced callus/No. of plants inoculated) X 100

Extraction of callus

Fresh and dry biomass weight was found to be higher in the combination of 1.5mg/L 2,4-D + 1.5mg/L IAA, that combination was used for mass cultivation of the callus and the same was used for further experiments. The methanol solvent was used for the extraction of callus. These callus extract used for the treatment for antiulcer activity.

Animal selection and standards used

Adult wistar albino rats 12-16 weeks male and female between 200 to 300g were used for this study. The animals in huge polypropylene cages were monitored in well ventilated room temperature with regular day-night cycle. Right through the experimental period, they were supplemented with an altruistic diet of rodent pellets and water *ad libitum*. The animals were quarantined for 1 week, to get used laboratory conditions before the experiments. Institutional Animal Ethics Committee, Govt. of India (IAEC) implemented the research protocol. For the standard negative control Vehicle - carboxy methyl cellulose (CMC) (Group I) and positive control (Group- II) omeprazole (antiulcer drug): 40 mg/kg body weight (b.w.) was used for this study (Table 2).

Acute oral toxicity study

The acute toxicity study was conducted according to the guidelines set by organization for economic co-operation and development (OECD) revised draft guidelines 423 B ("Up and Down" method) arriving from committee for the reason of control and supervision of experiments on animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India [10].

Preparation of animals and dose condition

In the temperature investigational animal room was kept 22±30C. Artificially lighting was maintained for 12:12 h light:dark cycle. The animals were provided with normal rat chow and distilled water *ad libitum*. The animals had been erratically picked and placed in their cages for a minimum 5 days before dosing to allow adaptation on to the laboratory environment. All the doses were prepared in CMC as suspending agent. In each cases the concentrations were prepared in 0.5 ml/100g of b.w.

Investigative substances were administered after fasting for 3 to 4 h in a single dose with stomach intubation. The methanol extract of callus 2000 LD50 Cut-Off mg/kg, body weight the vehicle CMC was used (1/10th of the LD50 doses were taken as therapeutic dose for successive anti-ulcer activity).

Animals and dose levels

In each step six animals was used. While no information was available on the drug to be tested (i.e. extracts), preliminary dose 50 mg/kg b.w. was chosen. Ever there was on the substance to be tested (extracts), selected to be the subsequent LD50 values were obtained for various extracts. Observation was carried out normal activity was note it down.

Pylorus Ligation Induced Gastric Ulcers

Animals were divided into six groups of six animals. Plant extracts were orally administered once a day for 7 days at the dosage of 200 mg/kg b.w. Aspirin was dissolved in 1% CMC solution and given orally for 6 days in non fasted rats once daily at a dosage of 200mg/kg. The animals were orally treated with methanolic callus extract of *J. Sambac* (Group III), at concentrations of 200 mg/kg b.w.

Omeprazole and callus extracts were administered to various behavioural groups 30 minutes before each aspirin treatment when only were provided by the control group (CMC 1% solution). On the 7th day, pylorus ligation was performed on 36 h fasted rats under ether anaesthesia, instantaneously after diagnosis with pylorus ligation aspirin treatment. Drinking water was withheld later than pylorus ligation was approved on the 7th day in each rat and gastric juice accumulated for a time of 4 hours [11].

After 4 h drug administration, pyloric ligation was performed by ligating end of animal stomach. Under chloroform anaesthesia illumination, the abdomen was opened below the xiphoid process by midline incision. The pyloric stomach section was pulled out and ligated somewhat, thereby preventing disruption to its blood supply. The stomach was carefully positioned back, and sutures closed the abdominal wall. Animals were placed in individual cages to heal and stabilize, and were deprived of water during the post-operative period. The animals were sacrificed by cervical displacement 4 hours later; the abdomen was opened and a further ligature was placed at the oesophageal end [12].

Collection of Gastric Juice, pH and Ulcer Score

The stomachs were separated, gathering and centrifuging the gastric substance for 10 min at 3000 rpm. Centrifuged gastric juice was collected and its volume as well as pH was measured. Ulcer score was calculated and expressed as ulcer index [13] method.

Ulcer percentage inhibition of was calculated as below:

$$\% \text{ Inhibition of Ulceration} = \frac{(\text{Ulcer index Control} - \text{Ulcer index Test})}{\text{Ulcer index Control}} \times 100$$

The collected gastric liquid was subjected to bio-chemical determination of free and total acidity in gastric juice [14], estimation of pepsin [15], estimation of proteins [16], estimation of total carbohydrates (hexoses, hexosamine and fucose)[17] were also calculated.

Statistical Analysis

The data obtained from these experiments were subjected to statistical analysis by using the statistical software AGRES, in completely randomized design (CRD). Each experiment was repeated twice with a minimum of three replicates in each. The SEM (Standard Error of Mean), Critical Difference (CD) and Co-efficient of Variation (CV %) values were calculated. Significant differences (p value <0.05) were determined using Duncan's multiple range test (DMRT).

RESULT AND DISCUSSION

Jasminum sambac (Table.1) plant percentage of callus formation highly observed in has shown higher MS media supplemented with single hormone of 1.5 mg/L 2,4-D (63.33 ± 3.21), fresh weight (665.33 ± 2.52) and dry weight (365.52 ± 1.78). Percentage of callus formation was the highest in 2,4-D followed by IAA, IBA and NAA, respectively.

In combination MS media supplemented with 1.5 mg/L 2,4-D+1.5 mg/L IAA (Figure 2) showed the highest activity in callus formation (91.00 ± 2.00). Similarly, fresh weight (992.67 ± 1.53) and dry weight (548.48 ± 3.53) was observed in the same hormone concentration. The friable callus was observed in single and combination of the hormone concentration.

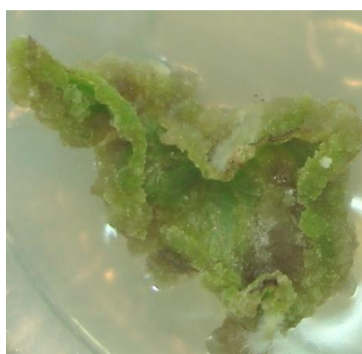


Figure 2. *J. sambac* callus

Table 1. Effect of various concentrations of auxin on callus initiation, callus formation, fresh and dry weight of *J. sambac*

Treatment (mg/L)	Callus initiation (Days)	Texture of the callus	% of callus formation	Weight (mg)	
				Fresh	Dry
2,4-D					
0.5	11-14	C	34.67±1.53 ⁱ	353.33±2.08 ^u	190.74±1.96 ^p
1.0	11-14	C	53.33±2.52 ^s	447.33±1.53 ^k	244.97±1.43 ^m
1.5	11-14	C	63.33±3.21^{cd}	665.33±2.52^c	365.52±1.78^d
2.0	11-14	C	59.67±3.06 ^{de}	544.09±2.87 ^h	303.96±3.76 ⁱ
IAA					
0.5	8-12	F	20.00±3.61 ^m	215.00±2.65 ^u	119.65±1.55 ^w
1.0	8-12	F	35.67±2.52 ⁱ	415.67±2.52 ^l	228.11±1.53 ⁿ
1.5	8-12	F	30.00±2.65 ^{jk}	296.67±1.53 ^q	163.99±1.77 ^s
2.0	8-12	F	26.33±2.52 ^{klm}	238.33±2.08 ^t	133.64±1.75 ^u
IBA					
0.5	12-15	C	18.00±1.00 ^o	235.67±2.52 ⁱ	128.19±0.74 ^v
1.0	12-15	C	26.67±2.52 ^l	313.00±2.65 ^p	174.61±1.67 ^r
1.5	12-15	C	34.33±4.16 ^{ij}	333.33±2.08 ^o	183.03±1.01 ^q
2.0	12-15	C	24.00±3.00 ^{lm}	363.67±2.52 ^m	195.76±2.42 ^o
NAA					
0.5	10-14	C	17.33±2.08 ^o	196.33±2.52 ^v	108.53±0.83 ^x
1.0	10-14	C	23.67±3.06 ^{lmn}	246.67±2.08 ^s	135.22±1.60 ^p
1.5	10-14	C	33.12±2.52 ^{ij}	315.67±1.53 ^p	175.10±1.38 ^r
2.0	10-14	C	19.33±3.51 ^{no}	250.67±2.08 ^t	140.91±1.72 ⁱ
2,4-D+ IAA					
1.5+0.5	9-11	F	66.33±3.06 ^c	578.41±2.50 ^f	320.64±1.57 ^e
1.5+1.0	9-11	F	75.67±3.51 ^b	785.67±3.06 ^b	429.64±3.05 ^c
1.5+1.5	9-11	F	91.00±2.00^a	992.67±1.53^a	548.48±3.53^a
1.5+2.0	9-11	F	68.00±2.00 ^{cd}	657.67±3.21 ^d	359.65±1.47 ^e
2,4-D+ IBA					
1.5+0.5	10-13	C	46.33±3.06 ^h	448.67±2.52 ^k	248.33±1.07 ^l
1.5+1.0	10-13	C	54.33±4.16 ^g	562.00±1.73 ^e	312.80±3.18 ^h
1.5+1.5	10-13	C	74.67±4.04 ^b	784.00±3.61 ^b	432.73±2.55 ^b
1.5+2.0	10-13	C	64.33±3.21 ^c	658.79±2.32 ^d	363.61±1.32 ^d
2,4-D+ NAA					
1.5+0.5	10-12	C	36.67±2.52 ⁱ	428.75±1.39 ^k	245.04±1.35 ^m
1.5+1.0	10-12	C	43.00±3.00 ^h	493.44±2.37 ^j	271.88±1.31 ^k
1.5+1.5	10-12	C	58.67±2.52 ^{ef}	592.33±1.53 ^e	326.43±1.82 ^f
1.5+2.0	10-12	C	51.33±4.51 ^g	517.21±2.55 ⁱ	284.25±1.98 ^j
CD (0.05)			4.5181	3.7516	2.9058
SEM			2.2535	1.8712	1.4494
CV%			6.20	0.50	0.70

C- Compact callus F-Friable callus Data are expressed as mean ± SD of three replicates followed by a common superscript letter are significant at 5% level by using DMRT (p<0.05). 2,4-D), Indole- 3-Acetic acid, (IAA), Indole-3-Butyric Acid (IBA), Naphthalene Acetic Acid (NAA)

Preliminary Screening and Estimation of LD₅₀

Acute toxicity study was carried out according to (up and down method) the method in albino rats. The LD₅₀ of *Jasminum sambac* callus extracts (200 mg/kg b.w) was taken as therapeutic dose and test extracts were administered in different groups higher dose level fixed 2000 mg/kg but no significant changes were observed.

Ulcer Score

Animals in this study indicated a significant ($p < 0.05$) raise in ulcer index and acid secretory parameters like pH, gastric volume, total, free acidity and perforated ulcers when compared with those of vehicle treated group. Administration of *Jasminum sambac* callus produced significant ($p < 0.05$) decrease in ulcer index. The callus extract also comprehensively decreased the gastric volume, free and total acidity, and improved the pH of the gastric fluid, proving its antisecretory activity.

Jasminumsambac callus (Figure. 3 a-c) at a dose of 200 mg/kg body weight indicated ulcer score of 1.01 ± 0.08 and protection index of 65.41% in maximum level followed compare to control. The pyloric ligation induced a control to accrete 5.17 ± 0.05 ml gastric secretions with a pH of 2.22 ± 0.05 . The total and free acidity of the gastric secretions were found to be 120.47 ± 0.46 and 66.44 ± 0.65 mEq/l respectively. In positive control (omeprazole) gastric acid secretions were low (2.30 ± 0.04) at a pH of 5.23 ± 0.08 . Free acidity was observed to be 26.45 ± 0.51 while total acidity was 54.51 ± 0.41 (Table 2). Treatment with callus extracts, significantly reduced the volume of gastric secretions at the dose of 200 mg/kg. Similar effect was observed as with positive control in *Jasminumsambac* callus which showed gastric volume of 2.66 ± 0.06 , pH 3.97 ± 0.05 , free acidity 36.19 ± 0.38 and total acidity 74.35 ± 0.29 . The gastroprotection accessible by the test extract was similar to that of the standard drug, omeprazole (40 mg/kg).

The pepsin content was drastically decreased in with the plant extracts when compared with the control group. The pepsin content was high in control group (9.17 ± 0.41) when compared to the positive control (4.60 ± 0.40). *Jasminumsambac* callus showed slightly higher content (6.49 ± 0.50).

Total carbohydrate (total of hexose, hexosamine and fucose) / protein content ratio (TC / PR) were considerably increased when compared with the control groups (Table 2). Very low carbohydrate: protein ratio was observed in control (0.43) when compared to the (standard- omeprazole) positive control (1.35). Treatment resulted in higher activity in *Jasminumsambac* callus (0.68) in 200mg/kg dose.

In vitro derived callus formation maximum observed in 2,4-D similarly in combination of 2,4-D only shown high callus formation in *Jasminum sambac*. Similar hormone combination has shown higher activity in *Jasminum grandiflorum* plant [9,18].

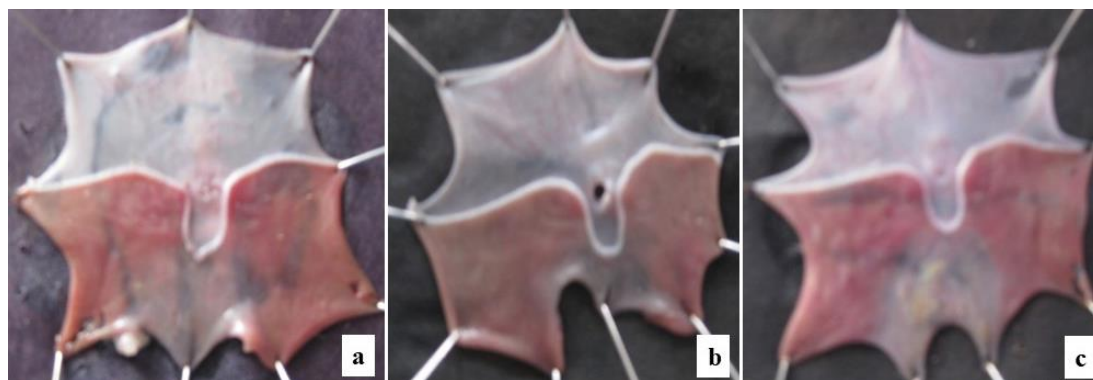


Figure 3. a. Control ulcer, b. Positive control ulcer, c. *J. sambac* ulcer

Table 2. Antiulcer activity of *J. sambac*

Experiments	Control (Group I)	Standard (Group II)	Dosage (mg/Kg 200) (Group III)
% Ulcer Inhibition	0.00	95.21	65.41
Ulcer score	2.92±0.06	0.14±0.02	1.01±0.08
Gastric Volume (ml)	5.17±0.05	2.30±0.04	2.66±0.06
pH	2.22±0.05	5.23±0.08	3.97±0.05
Free Acidity (mg/l)	66.44±0.65	26.45±0.51	36.19±0.38
Total Acidity (mg/l)	120.47±0.46	54.51±0.41	74.35±0.29
Pepsin (µg/ml)	9.17±0.41	4.60±0.40	6.49±0.50
Protein (µg/ml)	628.51±0.56	323.06±0.66	431.95±0.75
Hexose (µg/ml)	186.71±0.84	327.11±0.70	273.45±0.56
HexoSamine (µg/ml)	224.38±0.71	390.85±0.86	320.30±0.50
Fucose (µg/ml)	54.31±0.59	31.44±0.53	42.59±0.54
C:P ratio	1.35	0.43	0.68

Data are expressed as mean ± SED of six replicates

The causes of gastric ulcer are whispered due to stress triggered the addition of hydrochloric acid secretion and/or stasis, and the rate discharge is also an important reason in the ulcer configuration due to the exposure of the stomach's undefended lumen to the accumulating acid [19].

Ulcers are also induced by auto-absorption of the stomach mucosa and breakdown of the stomach mucosal barrier. Such causes are associated with the spread of upper gastrointestinal injury, as well as lesions, ulcers and life-aggressive damage and hemorrhage. Prostaglandin also induces the release of hydrophobic surfactant like phospholipids in the gastric epithelial cells. Volume of gastric secretion is an important factor in ulcer production due to exposure of unprotected stomach lumen to the accumulating acid [20].

The increased activity of the extracts needed in vitro pepsin that suggest a possible and significant in vivo binding of pepsin to substrate proteins through a non-specific hydrophobic interface to form complexes less susceptible to peptic hydrolysis. Although, this is only a hypothesis to which further studies could shed more light. There is strong correlation between the enrichment offered by the extracts and the mucous discharge adjacent to experimental ulcers. It is recognized that the mucus layer that covers the gastrointestinal tract wall is threaten, gastric mucous production may increase. The biochemical characteristics of the component mucin molecules can change in accumulation [21].

Because of the enlargement in acid pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion, pylorus ligation induced gastric ulcers transpire. The sufficient amount of mucus is secreted all through surface damage and provides desirable repair microenvironment. Mucin is an acid barrier which produces viscous glycoprotein, which is comparatively resistant. It constitutes a most important part of the mucus, an important pre-epithelial factor which acts as a first line of defense against ulcerative agents. The increase in mucin is due to significant increase in the individual mucopolysaccharide like sialic acid and total hexoses, which result in a significant increase in carbohydrates altogether. The plant fraction also significantly increase the comfortable glycoprotein of mucosal cells as seen from the increase of the gastric mucosa in the TC:PC ratio [22].

Peptic ulcer is the most common chronic digestive system disease that gives rise to symptoms of upper fullness, abdominal pain, bloating and gas. It may also cause serious problems in a few patients in a few patients, such as bleeding, perforation of the bowel and obstruction. Ulcer bleeding, a general explanation for admission to an emergency hospital, is the basis of most laboratory assessment of local plant antiulcer activity. There are many plants known to have and extended history of use in the digestive tract for calming inflamed and injured mucous membranes. Licorice, for example, may defend the stomach and duodenum by increasing mucin production. In addition, it has been shown that various active components of plants present antiulcer properties on some plants. Accordingly, experimental research reports have shown that compounds such as flavonoids can lower growth other than its direct cytoprotective effects on *Helicobacter pylori* [23,24].

The results of this study showed that the surveyed extracts of the investigated callus exhibited potent antiulcerogenic and dose related activity. Moreover, the methanolic extract of *Jasminum grandiflorum* produced a more effective cytoprotection to the mucosa [25,26]. It is obvious from this study that the plant callus extracts investigated are promising and effective research areas. The antiulcer activity of the investigated callus extracts tested again an antiulcer was substantially equal to the antiulcer drug activity. This is likely to lead to the development of a better drug with minimal side effect, much more efficient than the current antiulcer drugs.

In conclusion, our findings suggest that in vitro derived callus can be helpful to control ulcer in low dose level. Callus provides the source number of drugs formation without interruption of nature

plants. So it can provide drugs without destroying the nature plant and high amount of plant harvest from the environment with maintain the natural habitation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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