ANTIULCER ACTIVITY OF ETHANOL EXTRACT OF LEAVES OF *"BAMBUSA VULGARIS" (BAMBOO)* AGAINST INDOMETHACIN – INDUCED PEPTIC ULCER IN RAT.

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Abstract:

Aim of The Study: Bambusa vulgaris Schrad. Ex J. C Wendl. (Poaceae), a rhizomatous plant commonly known as Golden Bamboo, is widely distributed and grows in tropical and sub tropical areas. It is cultivated extensively in many parts of the world especially in wild fields and wet tropics. Bamboo leave have been reported to be used as Astringent, Ophthalmic solution and febrifuge. Ethane botanically, Bambusa vulgaris is use in traditional medicine for the treatment of several disease (e.g. measles), as an abortifacient, an appetizer and for managing respiratory diseases. The leaves have also been used in India for treatment of various inflammatory conditions. The present research was aimed to evaluate the potential antiulcer activity of aqueous extracts of leaves in vivo models to validate to folkloric use of the plant.

Materials and Method: The antiulcer activity of aqueous extracts of leave were studied on Female albino wistar rat, stomach damage induced by Indomethacin (20mg/kg) all the chemical, solvent and reagent used were of AR grade and were purchased from Tunnex chemicals, at the herbarium of Botany Department, University. The antiulcer activity of aqueous extracts of leaves were stuthe collected plant was identified and authenticated by the Botanical Survey in India, the collected leaves of Bambusa vulgaris were dried in shade at room temperature and reduced to course powder using a mechanical grinder. The dried powder material was extracted with Petroleum ether (60-8 0C) by Hot continuous extraction in a Soxhlet,s s apparatus for 48 h. The extract was concentrated under reduced pressure and stored in an air tight container. Histopathological change in stomach were also studies along with Ranitidine (20mg/kg) as standard antiulcer agent were determine to assess the effect of the aqueous extract of leave of Bambusa vulgaris (100, 150 and 200mg /kg) on the Indomethacin induced peptic damage .

Result: The phytochemical investigation of the extract showed presence of carbohydrates, steroids and flavonoids. Pre- treatment of the rat with aqueous extract prior to Indomethacin administration, all the extracts showed the decrease in gastric juice. Volume on comparison to control group and indicated their anti-secretary effort. But 90% Aqueous extract (200mg/kg) showed significant effect on that of ranitidine (20mg/kg) in reducing the gastric juice volume. Compared the controlled group all the test extract showed elevation in pH indicating their capacity to reduce the acidity of the gastric juice, the 90% Aqueous extract At 200mg/kg indicate almost equipotent effect on that of Ranitidine. Gastric free acidity is increased in control animal due to ulcer.

Conclusion: The result indicates that this plant possesses potential antiulcer properties and has therapeutic potential for the treatment of stomach diseases.

***Keywords*:** Bambusa vulgaris, Indomethacin, Antiulcer Activity, Ranitidine.

I - Introduction

Herbal medicine have recently attached much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action . There has been growing interest in the analysis of plant product which has stimulated intense research on their potential health benefits. In the present study we selected a plant namely *Bambusa vulgaris* belongs to the family Poaceae. It is one such plant used by the tribes and native medical practitioners. Bamboo is the name given altogether 1575 species of perennial evergreen plant that belongs to the subfamily *Bambusoideae* of the true grass family Poaceae. *Bamboo* is naturally distributed throughout the globe. The *Bamboo* leaf extract (BLE) is thought to be good source of natural antioxidant and also have great pharmaceutical potential. BLE is mainly composed of flavonoids, lactones and Phenolic acid. The flavonoids are represntely mainly by the flavones C- glycosides. Which include homoorientin, isovitexin, orientin and vitexin, apart from this quercetin Luteolin, caffeine acid, chlorogenic acid and tricin are also present. The flavonoids content was recorded to be 3.44% in different bamboo leaves species. The use of Bamboo as a traditional medicine by the Chinese dates back to some 2500 year. They used the Bamboo leaves, branches, shoots, seeds roots and juice to treat phlegm, cooling fever. Laryngitis, rhinorrhagia and vomiting. Thus it can safely be asserted that each part of bamboo is not only a treasure but also a medicine. It is cultivated extensively in many part or world especially in
Wild field and wet tropics. *Bambusa vulgaris* from bright green dense tufted culms and grow 10-20cm (30-70ft) high and 4-10 cm in diameter with thickness range 7-15mm. the plant locally called “Oparun” is used for the rural construction of houses , huts, boats fences, furniture, musical instrument but user are restricted to the culm and not the leaves.

On the basis of literature review and tribal information to gathered from Allahabad interior area,UttarPradesh that the plant *Bambusa vulgaris* (Golden Bamboo) has the reported the use of both leaf for the management of ulcer toxicity. Hence the objective of this study was to ascertain the scientific basis for the use of *Bambusa vulgaris* in the management of ulcer toxicity using Indomethacin induced ulcer toxicity rat

Fig 1- Plant of *Bambusa vulgaris*

II- Material and Methods

2.1- Plant Material

The leave of *Bambusa vulgaris* was collected from the local area of Jhalwa, Allahabad, and Uttar Pradesh, India in the month of Sep 2016 and the plant specimen is authenticated by "***BOTANICAL SURVEY OF INDIA, ALLAHABAD***". Ref. No-97112. The collected leaves of Bambusa vulgaris were dried in shade at room temperature and reduce to course powder and reduce the course powder using a mechanical grinder. The dried powder material was extracted with petroleum ether by hot continuous extraction in a Soxhlet apparatus for 48 h. The extract was concentrated under reduce pressure and stored in air tight container.

2.2- Extraction

The leaves were separated from the fresh stems and dried on filter paper sheet under shade at room temp until with changing of color of filter papers. The shade dried, coarsely. Powdered leaves (500g) were successively extracted with Petroleum ether (60-80 oC) for 8 hr. to remove fatty matter. The defatted marc was then subjected to Soxhlet,s, s extraction with 95% methanol to obtain Aqueous extract. The Aqueous extract were evaporated under reduce pressure at low temp (30 0C) to dryness to yield brownish yellow color extract of *B. vulgaris* store in an air tight container in refrigerator for further experimental studies.

Fig 2- Crude powder leaves of extract (Bamboo)

III- Preliminary phytochemical Screening

The aqueous extract of leaves of *Bambusa vulgaris* was screened for the presence of various phytoconstituents like steroids, alkaloids, tannins, flavonoids, and glycoside, by employing standard phytochemical tests.

Table 1: Preliminary phytochemical study of the extracts of leaves of *Bambusa vulgaris*

Phytoconstituents/Extract	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
Alkaloids	+	+	+	+	+
Glycosides	+	+	+	+	+
Flavonoids	-	+	+	+	+
Steroids	-	-	-	-	-
Phenolic and Tannins	-	+	+	-	+

+ = Presence; - = Absence

IV- Animal: - Female albino rat of wistar weighing around 160-180g were procured from CDRI. They were acclimatized to animal house condition, fed with commercial pellet raw chow and free access the water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) Regd. No SIP IAEC/005/09/16.

Fig 3- Animal divided in groups of study

V- Test Compound Formulation

The aqueous suspension of extract of leaves of *Bambusa vulgaris* was prepared in 0.5% carboxyl methylcellulose (CMC) solution in distilled water prior to oral administration to animal.

It was used within one days and store at 8oC while for further use freshly prepared solution was used. The vehicle alone served as control.

VI- Chemical: - All the drugs and chemical were of analytical grade. Ranitidine (Osaka) were used.

4.1- Acute Toxicity Studies

Acute Toxicity Studies were performed according to organization for economic co-operation and development guideline. Animals were divided in groups (n=5). The animals were fasted for overnight, with free access to water only.

V- Antiulcer Activity – Indomethacin Induced Ulcer in Rat

5.1- Purpose and Rational

Nonsteroidal anti-inflammatory agent like Indomethacin and acetyl-salicylic acid, induced gastric lesion in man and in experimental animal by inhibition of gastric cyclooxygenase resulting in less formation of prostacyclion, the predominant prostanoid produced in the gastric mucosa.

5.2- Procedure

Groups of 5 wistar rats weighing 150-200gm are used. The test drugs are administered orally in 0.1% Tween 80 solution 10 min prior to oral Indomethacin in dose of 20 mg/kg (4mg/ml dissolve in 0.1% Tween 80 solution). Six hour later, the rats are sacrificed in Co2 anesthesia and their stomach removed.

Formal-saline (2%v/v) is then injected into the totally ligated stomach for storage overnight. The next day the stomachs are opened along the greater curvature, then waged in warm water and examined under a 3-fold magnifier. The length of the longest diameters of the lesion are measured and summated to give a total lesion score (in mm) for each animal, the mean count for each group being calculated.

5.3- Microscopic evaluation of stomach

The stomach were opened along the greater curvature, rinsed with saline to remove gastric content and blood clots and examined by a 10x magnifier lens to assess the formation of ulcer. The number of ulcer was counted.

5.4- Scoring of Ulcer Will Be Made As Follows:-

Normal colored stomach……. .(0)
Red coloration ……. (0.5)
Spot ulcer…… …… (1)
Hemorrhagic streak…….. (1.5)
Deep ulcer……. ……. (2)
Perforation…… ……. (3)
Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer
Protection was determined as
Fallows:-
Ulcer index (UI) was measured by using following formula:
UI = UN + US + UP X 10-1
Where
UI= Ulcer index, UN= Average number of ulcer per animal
US= Average number of severity score, UP= Percentage of animals with ulcer.
Percentage inhibition of ulceration was calculated as below:

$$\% \text{ inhibition of ulceration} = \frac{\text{Ulcer index control- Ulcer index test}) \times 100}{\text{Ulcer index control}}$$

Table 1: Effect of Aqueous extracts of *B. vulgaris* gastric content pH, total and free acidity in pyloric ligation induced ulceration in rats.

Treatment	Dose (mg/kg)	Gastric Content (ml)	pH	Acidity Total (mEq/l)	Free
Normal	-	-	-	-	-
Toxic Controlled (Distilled water)	**10**	**8.03±0.16**	**3.016±0.23**	**110.5±0.35**	**95.05±0.39**
Extract drug High dose Lower dose Medium dose	 **200** **100** **150**	 **4±0.37** **4.11±0.55** **3.14±0.17**	 **4.16±0.42** **3.68±0.34** **2.27±0.25**	 **65.62±0.38** **67.67±0.36** **44.71±0.29**	 **66.77±0.32** **70.09±0.38** **39.65±0.31**
Standard (Ranitidine)	**20**	**4.03±0.36**	**5.15±0.23**	**2.49±0.38**	**36.93±0.55**

Value are expressed as (Mean +-S. E. M.), n=6, *p > 0.10 when compared with control group

VI- Determination of pH

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using pH meter.

VII- Determination of total acidity: -

An aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink color was observed. The volume of 0.01N NaOH consumed was noted. The total acidity is expressed as mEq/L by the following formula:

$$\text{Acidity} = \frac{\text{Vol. of NaOH x N x100 mEq/L}}{0.1}$$

VIII- Determination of Free Acidity

Instead of phenolphthalein indicator, the Topfer's reagent was used. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow color was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity.

IX- Statistical Analysis

The results are expressed as the mean +_SD for each group. Statistical difference were evaluated using a One –way analysis of variance (ANOVA) followed by Dun net's t- test.
Result were considered to be statistically significant at p>0.0.

Table 2: Effect of Aqueous extract of *B. vulgaris* on Peptic ulcer induced by pylorus ligation in rats.

Treatment	Dose (ml/kg)	Ulcer Index	% Ulcer Inhibition
Normal	-	-	-
Toxic controlled (distilled water)	**10**	**3.63±0.29**	-
Extracted drug High	**200**	**2.54±0.19**	**30.027**
Lower	**100**	**2.48±0.17**	**31.68**
Medium	**150**	**2.36±0.13**	**13.77**
Standard (Ranitidine)	**20**	**1.34 ±0.21**	**63.085**

Value are expressed as (Mean +- S.E.M), n=6 *p > 0.10 when compared with control group.
(Statistically analyzed by one –way analysis of variance (ANOVA) followed by Dun net's test

Fig1- Normal dose

Fig2- Toxic dose

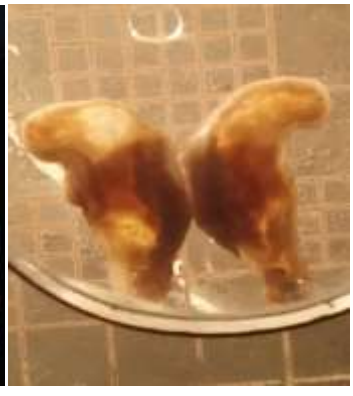

Fig3-Standard dose


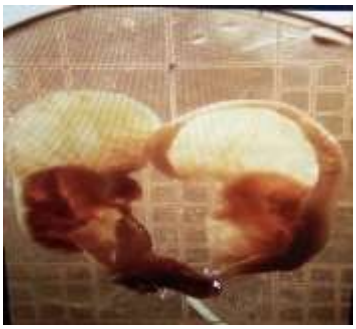


Fig4- High dose **Fig 5- Low dose** **Fig6- Medium dose**

Figures of rat's stomach show the ulcer

X- Results

Effect of ethanol extract of B.vulgaris on pyloric ligation induced ulceration is shown in Table1. The pyloric ligation has caused the accumulation of gastric secretions of 8.03± 0.16 MI with pH 3.016± 0.23 in a control group. The total acidity and free acidity of the gastric secretion were found to be 110.5 ±0.35 mEq/l respectively. Pre – treatment with the B. vulgaris extract significantly (p>0.010) reduce the volume of gastric secretions 4.11±0.55 and 4±0.37 ml at the doses of 100 and 200mg/kg respectively. pH of the gastric fluid was significantly (p>0.010) elevated up to 4.1 ±0.55 only at higher doses of the extract in addition total acidity and free acidity were also reduce significantly (p>0.010) in a dose dependent manner. Further it is observed that pyloric ligation has caused gastric ulceration and pre- treatment with B. vulgaris extract has reduced them significantly (p>0.010) in a dose dependent manner. In this model, percentage inhibition of ulceration was found to be 31.68 and 30.027 at 100 and 200 mg/kg respectively. , the stomach protection offered by the test extract was comparable to that to the standard drug; Ranitidine (20 mg /kg).

XI- Discussion

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factor and the maintenance of mucosal integrity through the endogenous defense mechanism. To regain the balance, different therapeutic agents including plant extract may be used. *B. vulgaris* extract is one such Herbal drug used in the present study primarily to evaluate the anti-ulcerogenic and ethanol induced ulcer in rat. the causes of peptic ulcer are believed to be due to stress induced increase in peptic hydrochloric acid secretion and/ or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid. Peptic induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. The factors are associated with the development of upper gastrointestinal damage including lesion, ulcer and life threatening perforation and hemorrhage. Aspirin. Phenybutazone, Indomethacin and some non-steroidal anti-inflammatory drug are also known to cause duodenal and peptic ulceration. Ethanol is also has been reported to cause disturbances in gastric secretion, damage to the mucosa, alteration in the permeability, gastric mucus depletion and free radical production. This is attributed to the release of superoxide anion.

Hydroperoxy free radical during metabolism of ethanol as oxygen derived free radical has been found to be involved in the mechanism of acute and chronic ulceration in the gastric. This may be due to cytoprotective effect of the extract via antioxidant effect. The extract shown protection against characteristic lesion produced be ethanol administration this antiulcer effect of MEAI may be due to both reduction in gastric acid secretion and stomach cytoprotection. The antiulcer activity of B.vulgaris in peptic ulcer model is evident from its significant reduction in free acidity, total acidity, number of ulcer and ulcer index. *B.*

vulgaris treated animals significantly inhibited the formation of ulcer in the peptic ulcer rats and also decreased both the conc. and the increased the pH. The preliminary phytochemical analysis of B. vulgaris extract showed the presence of alkaloid, flavonoids triterpenoids, carbohydrates, and glycosides. The significant increase in the antiulcer activity of B. Vulgaris could be attributed to the presence of flavonoids, alkaloids, tannins, saponin glycoside and Phenolic compounds. So the antiulcer activity of *B .vulgaris* may be flavonoids content. The result of the present study suggests that the ethanol extract of B. vulgaris leaves may be beneficial in the treatment of peptic ulcer.

XII- Acknowledgement

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